California GAMA Program: Impact of Dairy Operations on Groundwater Quality

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Appendix B: McNab, W. W., Singleton, M. J., Moran, J. E., and Esser, B. K., 2007. Assessing the impact of animal waste lagoon seepage on the geochemistry of an underlying shallow aquifer. *Environmental Science & Technology* **41**, 753-758.

EXECUTIVE SUMMARY

A critical component of the California State Water Board's Groundwater Ambient Monitoring and Assessment (GAMA) Program is to assess the major threats to groundwater resources that supply drinking water to Californians (BELITZ et al., 2003). Nitrate is the most pervasive and intractable contaminant in California groundwater and is a focus of special studies under the GAMA program.

This report assesses the impact of Central Valley dairy operations on underlying groundwater quality and on groundwater processes using new tools developed during the course of the study. During the investigation, samples were collected and analyzed from a total of five dairies in the San Joaquin-Tulare Basins of California: three in Kings County, one in Stanislaus County, and one in Merced County (Figure 1). The study investigated water samples from production wells, monitor wells, and manure lagoons..

The three primary findings of this research are that dairy operations do impact underlying groundwater quality in California's San Joaquin Valley, that dairy operations also appear to drive denitrification of dairy-derived nitrate in these groundwaters, and that new methods are available for characterization of nitrate source, transport and fate in the saturated zone underlying dairy operations.

This study demonstrated groundwater quality impact at three sites using a multi-disciplinary approach, and developed a new tool for source attribution in dairy groundwater. Negative groundwater quality impacts from dairy-derived nitrate were demonstrated using groundwater chemistry, nitrate isotopic composition, groundwater age, and transport modeling. A significant advance in characterization of groundwaters for nitrate source determination was the use of groundwater dissolved gas content to distinguish dairy wastewater irrigation from dairy wastewater lagoon seepage, both of which contributed to dairy groundwater contamination.

The demonstration of saturated-zone denitrification in dairy groundwaters is important in assessing the net impact of dairy operations on groundwater quality. The extent of denitrification can be characterized by measuring "excess" nitrogen and nitrate isotopic composition while the location of denitrification can be determined using a bioassay for denitrifying bacteria that developed in this research. In both northern and southern San Joaquin Valley sites, saturated-zone denitrification occurs and mitigates the impact of nitrogen loading on groundwater quality.

Other new methods developed during the course of this study include the field determination of denitrification in groundwater (allowing siting of monitor wells and mapping of denitrifying zones) and characterization of aquifer heterogeneity using direct-push drilling and geostatistics (allowing development of more accurate groundwater transport models). Application of these new methods in conjunction with traditional hydrogeologic and agronomic methods will allow a more complete and accurate understanding of the source, transport and fate of dairy-derived nitrogen in the subsurface.

STUDY SITES: HYDROGEOLOGIC SETTING

Two concentrations of dairies exist in the Central Valley of California, which is a low relief structural basin that is from 60 to 100 km wide and 700 km long. Both centers are in the southern two-thirds of the basin - the northern concentration is in Merced and Stanislaus Counties, and the southern concentration is in Kings and Tulare Counties. Both concentrations of dairies occur in the San Joaquin Valley Groundwater Basin, as designated by the California Department of Water Resources (2003). The San Joaquin Valley groundwater basin comprises two of the Central Valley's three large structural sub-basins: the San Joaquin Basin and the Tulare Basin. In this document, we will use "San Joaquin Valley Basin" and "San Joaquin-Tulare Basin" interchangeably.

During the investigation, samples were collected and analyzed from a total of five dairies in the San Joaquin-Tulare Basins of California: three in Kings County, one in Stanislaus County, and one in Merced County (Figure 1). Groundwater samples were collected from production wells on each of the dairies. On three of the dairies, samples were also collected from monitoring wells: one of sites in Kings County was instrumented by LLNL, and the two sites in Stanislaus and Merced Counties were instrumented by UC-Davis. Samples were collected from manure lagoons at four of the sites.

Northern Sites

The two northern sites (SCD and MCD) are part of an extensive shallow groundwater monitoring network on five representative dairies set up by Thomas Harter of UC-Davis and the UC Cooperative Extension. The following description of the study area and the dairies is adapted from Harter et al. (2002).

The northern sites study area is in the central-eastern portion of the northern San Joaquin Valley, an area of low alluvial plains and fans bordered by the San Joaquin River to the west, tertiary upland terraces to the east, the Stanislaus River to the north, and the Merced River to the south. The region has a long history of nitrate and salt problems in groundwater (LOWRY, 1987; PAGE and BALDING, 1973).

The main regional aquifer is in the upper 100-200 m of basin deposits, which consist of Quaternary alluvial and fluvial deposits with some interbedded hardpan and lacustrine deposits. Groundwater generally flows from the ENE to the WSW following the slope of the landscape. The average regional hydraulic gradient ranges from approximately 0.05% to 0.15%. The water table at the selected facilities is between 2 and 5 m below ground surface. Measured K values range from 0.1 to 2 x 10^{-3} m/s, as consistent with the predominant texture of the shallow sediments.

The dominant surface soil texture is sandy loam to sand underlain by silty lenses, some of which are cemented with lime. Water holding capacity is low and water tables are locally high (and maintained by community drainage systems and shallow groundwater pumping). Border flood irrigation of forage crops has historically been the dominant cropping system among dairies in the study area. Low-salinity (0.1–0.2 μS/cm) surface water from the Sierra Nevada is the main source of irrigation water.

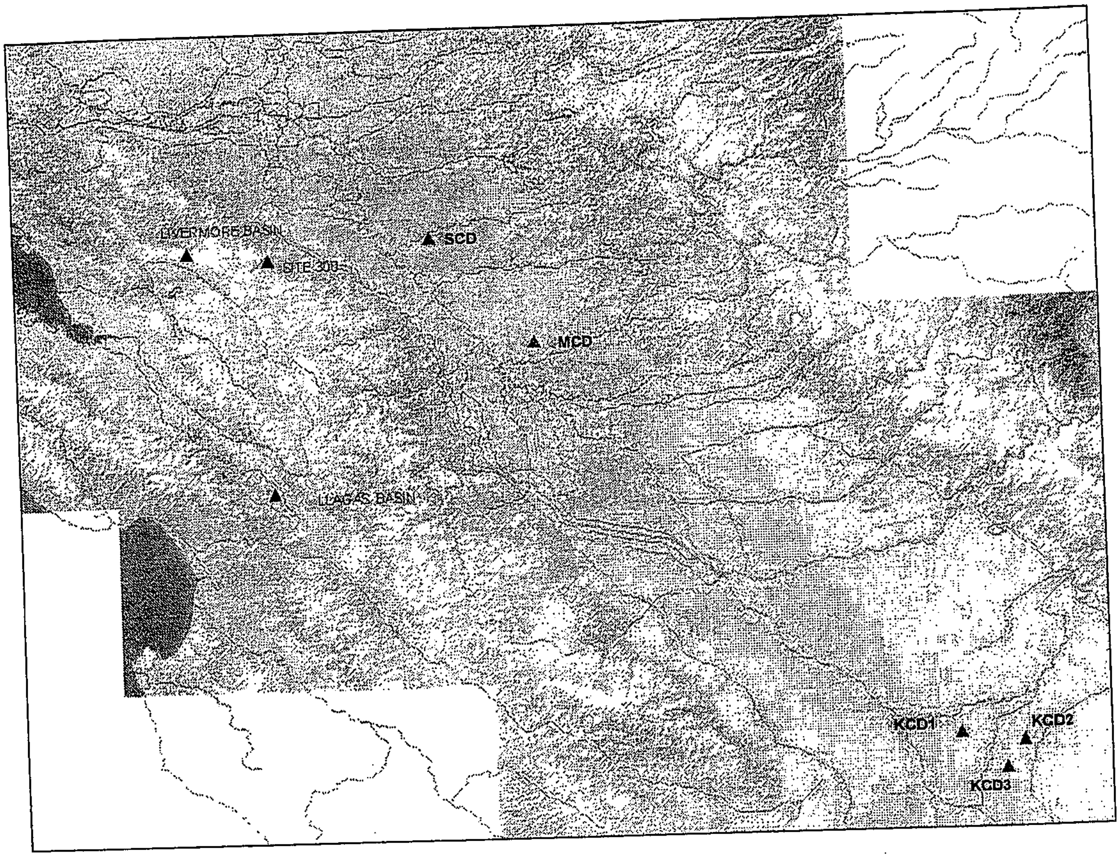

Figure 1. Dairy Field Sites in the Central Valley.

Dairy Field Sites in the Central Valley Dairy study sites in Kings County (KCD1, KCD2, and KCD3), Merced County (MCD) and Stanislaus County (SCD) are shown with red triangles. Other sites where LLNL has conducted groundwater nitrate studies are shown with blue triangles

A number of hydrogeologic criteria make the area suitable as a field laboratory for investigating recharge water quality from dairies: 1) Groundwater in the area is highly vulnerable because of the sandy soils with high infiltration rates and shallow water tables. 2) The shallow groundwater table and small long-term fluctuations in water level (1-2 m) allow sampling from vertically narrow groundwater zones with well-defined recharge source areas. 3) These same two factors also allow installation of a relatively inexpensive fixed-depth monitoring well network that is also inexpensive to sample.

The five dairy facilities in the UC-Davis network are progressive with respect to herd health, product quality, and overall operations. Improvements in manure and pond management have continually occurred since the inception of the project. The dairies are located in a geographic and hydrogeologic environment that is representative of many other dairies on the lowlands of the northern San Joaquin Valley. The manure management practices employed at these dairies over the past 35 years, particularly with respect to corral design, runoff capture, and lagoon management, have been recognized by industry, regulators, and university extension personnel as typical or even progressive relative to other California dairies (see references in HARTER et al., 2002). Over the past 30– 40 years, the herd size on these dairies has continually grown from less than 100 at their inception to over 1000 animal units in the 1990s.

In 1993, UC-Davis installed 6 to 12 monitoring wells on each dairy for a total of 44 wells. Monitoring wells are strategically placed upgradient and downgradient from fields receiving manure water, near wastewater lagoons (ponds), and in corrals, feedlots, and storage areas (henceforth referred to as ''corrals''). Wells are constructed with PVC pipe (3 or 5 cm diameter) and installed to depths of 7–10 m. The wells are screened from a depth of 2–3 m below ground surface to a depth of 10 m. Water samples collected from monitoring wells are representative of only the shallowest "first-encounter" groundwater.

Southern Sites

To augment the UC-Davis dairy monitoring network, LLNL chose to establish sites in the southern San Joaquin Valley groundwater basin. LLNL developed a list a five potential cooperators, sampled three sites, and chose to instrument one site. The cooperators were chosen with the expertise and assistance of the University of California Cooperative Extension (Thomas Harter, Carol Collar and Carol Frate). Sampling sites were chosen from the list of cooperator dairies using regional water quality data, including NAWQA data from the USGS and water quality dairy data from the Central Regional Water Quality Control Board (Fresno office). The site chosen for more extensive instrumentation was chosen with the following criteria: 1) a cooperative operator, 2) a shallow depth to groundwater to allow cost-effective installation of multi-level wells and synoptic soil-groundwater surveys, 3) a dairying operation typical for the region, and 4) regional evidence for nitrate contamination and denitrification.

The three dairies sampled are within the Tulare Lake Groundwater Subbasin of the San Joaquin Valley Groundwater Basin (CALIFORNIA DWR, 2003) (Figure 1). The sites are located south of the Kings River and north-northeast of the Tulare Lake basin, the natural internal drainage for this hydrologically closed system. Groundwater hydraulic gradients are regionally from the Kings River toward Tulare Lake, but are generally low and are locally influenced by recharge from unlined irrigation canals and by agricultural and municipal groundwater extraction. Surface soils at these sites are predominantly Nord series (USDA NATIONAL RESOURCE CONSERVATION SERVICE, 2006), and are developed on distal Kings River alluvial fan deposits (WEISSMANN et al., 2003; WEISSMANN et al., 1999; WEISSMANN and FOGG, 1999; WEISSMANN et al., 2002a), which in general are less sandy and have more fine-grained interbeds than the sediments in the northern UC-Davis monitoring network. Groundwater levels in the area are in general deeper (50-200' below ground surface) and more variable (50' over 2-5 years) than in the north. A deeper depth to groundwater and heavier textured soils indicate that southern groundwaters should be less vulnerable to contamination than northern groundwaters. The regional groundwater is highly impacted by agricultural activities and contains elevated concentrations of nitrate and pesticides (BUROW et al., 1998b; BURROW et al., 1998).

Two of the three dairies sampled (KCD2 and KCD3) have deep water tables typical of the region. The one dairy that LLNL instrumented is located in an area to the west of Hanford characterized by a shallow perched aquifer, with depth to groundwater on the order of 15 feet. California Department of Water Resources (DWR) water level data for wells in the area indicate that this perched aquifer developed in the mid-1960's in response to local groundwater overdrafting (CARLE et al., 2005), and is separated by an unsaturated zone from the deeper regional aquifer (that is sampled by wells on KCD2 and KCD3 to the east and south of Hanford).

The three dairy sites sampled by LLNL in Kings County each have close to the average of 1000 dairy cows, fed in free stalls with flush lanes. The manure management practices employed at these dairies, with respect to corral design, runoff capture, and lagoon management, are typical or progressive relative to other California dairies (see references in HARTER et al., 2002). The most intensively studied dairy, KCD1, operates three clay-lined wastewater lagoons that receive wastewater after solids separation. Wastewater is used for irrigation of 500 acres of forage crops (corn and alfalfa) on the dairy and on neighboring farms; dry manure is exported to neighboring farms. This dairy is also immediately adjacent to another dairy operation, and many of the conclusions regarding nitrate impact apply to dairy practices shared by both operations.

STUDY SITES: SAMPLING AND INSTRUMENTATION

Kings County Dairy Site 1 (KCD1)

Kings County Dairy #1 (KCD1; see Figure 1, Appendix A-Figure 1, and Appendix B-Figure 1), was the primary site in Kings County, and was sampled on multiple occasions, from existing production wells, from LLNL-installed monitor wells, from manure lagoons and irrigation canals, and with direct push soil and water sampling methods. A total of 31 days were devoted to collecting 139 water samples at the site, including 29 direct push samples, 17 surface water samples from 3 manure lagoons and a nearby irrigation canal, 16 groundwater samples from 9 production wells, and 60 groundwater samples from 17 monitor wells. A large number of subsurface soil samples were also collected, both as continuous drill core and as depth-discrete grab samples. Production and monitor wells were sampled on semi-regular intervals between August 2003 and August 2005.

KCD1 was instrumented with five sets of multi-level monitoring wells and one "up-gradient" well near an irrigation canal (Figure 2). The multi-level well "clusters" consisted of wells installed in separate boreholes approximately 5' apart. A first set of three nested 2" wells in one cluster was installed in September 2003. In August 2004, three new well clusters were installed, each with four 2" wells. Also at that time, an upgradient 2" well was installed, and a small cluster of three 1.25" wells were installed. Two aquifers underlie the KCD1 dairy site, a shallow perched aquifer and a more regionally extensive deep aquifer. The deep aquifer is instrumented with one 2" well screened at 178-180' below ground surface (bgs) that was installed in September 2003. The remaining monitor wells are all in the shallow perched aquifer and are screened between 18' and 65' bgs.

In August 2004, shortly before the second sets of well clusters were installed, a CPT/DP survey (see methods section) was conducted across the site (Figure 3). Depth discrete water and soils samples were collected at this time, after which the holes were grouted and abandoned. With the exception of the upgradient monitor well near the canal, CPT/DP sites included locations near all of the multi-level monitor well clusters.

Figure 2. KCD1 Dairy Field Site.
KCD1 site, showing monitor wells and direct-push locations. Sites 1, 2, 3, and 4 (S1 through S4) are all multi-level two-inch monitor well clusters; site 5 (S5) is a single two-inch first-encounter well. The Site 1 cluster (S1) also includes a well in the deep aquifer. Direct-push (DP) and cone penetrometer (CPT) holes are also shown. CPT/DP was done at all multi-level well sites; it was not done at the single-level 5S site. Inset shows application of manure lagoon wastewater for furrow irrigation of silage corn crops at the site.

The production wells are screened in both the shallow and deep aquifer, and have 20-30' long screens. Domestic supply wells, one of which was sampled, are screened in the deep aquifer, and typically have 20' long screens. Agricultural supply wells, eight of which were sampled, typically have 30' long screens, with the top of the screen at 30' bgs. Information on screen length and depth is from conversations with the water well company which installed the more recent wells and has extensive experience in the region.

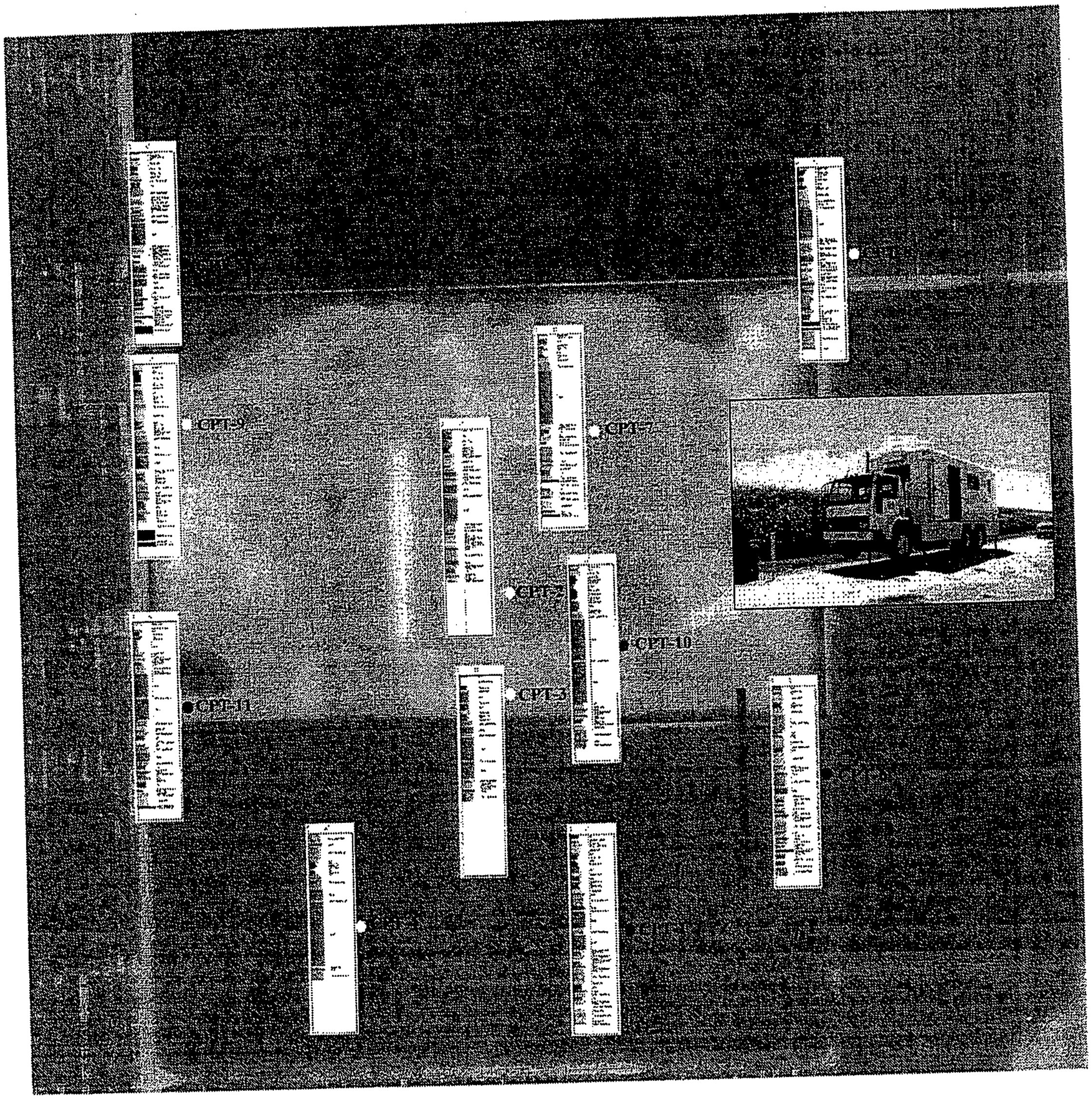

Figure 3. KCD1 field site with CPT/DP locations.
Soil Behavior Type (SBT) profiles from Direct-Push Cone Penetrometer Testing on the KCD1 dairy field site. Large inset shows direct-push rig.

Kings County Dairy Sites 2 and 3 (KCD2 and KCD3)

The second and third Kings County dairy sites (Figure 1) were sampled during initial screening of Kings County sites in August 2003. At each site, groundwater pumped from a domestic supply well was analyzed for inorganic cations and anions (including nitrate, nitrite and ammonia), dissolved gases by membrane-inlet mass spectrometry, and tritium/helium-3 mean groundwater age by noble gas mass spectrometry. Groundwater in the area is 120-150 feet below ground surface, and the Corcoran Clay is generally 400-450' below ground surface and 90-100' thick. At each site, groundwater was sampled from wells screened between 200 and 300 feet below ground surface.

The second dairy was sampled again in April 2005. On this occasion, groundwater from the same domestic supply well sampled in 2003 was re-sampled, and manure lagoon and field water from six sampling locations was sampled. The groundwater was analyzed as before; while the lagoon water samples were analyzed for inorganic cations and anions (including nitrate, nitrite and ammonia), and dissolved gases by membrane-inlet mass spectrometry.

Merced and Stanislaus Dairy Sites (MCD and SCD)

MCD and SCD (Figure 1, Appendix A-Figure 1: The Merced County and Stanislaus County Dairies (MCD and SCD) were sampled on three occasions: August 2003, April 2005 and June 2005. Almost 40 samples were taken broken down as follows: 30 MCD samples and 9 SCD samples; 28 groundwater samples from 22 wells, 1 lagoon water sample, and 1 tile drain sample. Groundwater samples were analyzed for field parameters (temperature, conductivity, dissolved oxygen and ORP); inorganic cations and anions (including nitrate, nitrite and ammonia), dissolved gases by membrane-inlet mass spectrometry, tritium/helium-3 mean groundwater age by noble gas mass spectrometry, stable isotopic composition of nitrate and water, and organic co-contaminants. Tritium/helium-3 samples were not taken from the surface water sampling sites. These sites and data from these sites are described in Harter et al. (2002)

METHODS

Cone Penetrometer (CPT) and Direct Push (DP) Methods

Standard cone penetrometer/direct push methods were used to characterize the shallow hydrostratigraphy at the site. The survey was accomplished using a 20-25 ton CPT rig and accompanying support rig. The dead weight of the CPT rig was used to push the cone penetrometer to depths up to 90 feet using a hydraulic ram located at the center of the truck. Soil parameters such as cone bearing, sleeve friction, friction ratio and pore water pressure were measured as the cone penetrometer was advanced. These measurements were sent through the cone rods to the CPT rig's on-board data acquisition system. All data was processed in real time in the field, and CPT plots of tip resistance, sleeve friction; friction ratio and pore pressure were provided in the field along with a table of interpreted soil parameters. For development of geostatistical models of subsurface hydraulic properties, soil behavior types determined by CPT (ROBERTSON et al., 1983) were calibrated and validated against a 200-foot continuous core log recovered from the first site (Figure 4.)

After CPT logging, a second hole was developed for collecting depth-discrete groundwater and soil samples using direct push methods. For water, a Hydropunch groundwater sample was taken at specified depth intervals. The Hydropunch operates by pushing 1.75-inch diameter hollow rods with a steel tip. A filter screen is attached to the tip. At the desired sampling depth, the rods are retracted, exposing the filter screen and allowing for groundwater infiltration. A small diameter bailer is then used to collect groundwater samples through the hollow rod. Typically, 4 or more 40 ml VOA vials were collected. For soil, a piston-type soil sampler was used to collect undisturbed soil samples (12" long x 1" diameter) that were stored on ice or dry ice immediately upon retrieval. After completion of logging and sampling, CPT/DP sampling holes were grouted under pressure with bentonite using the support rig.

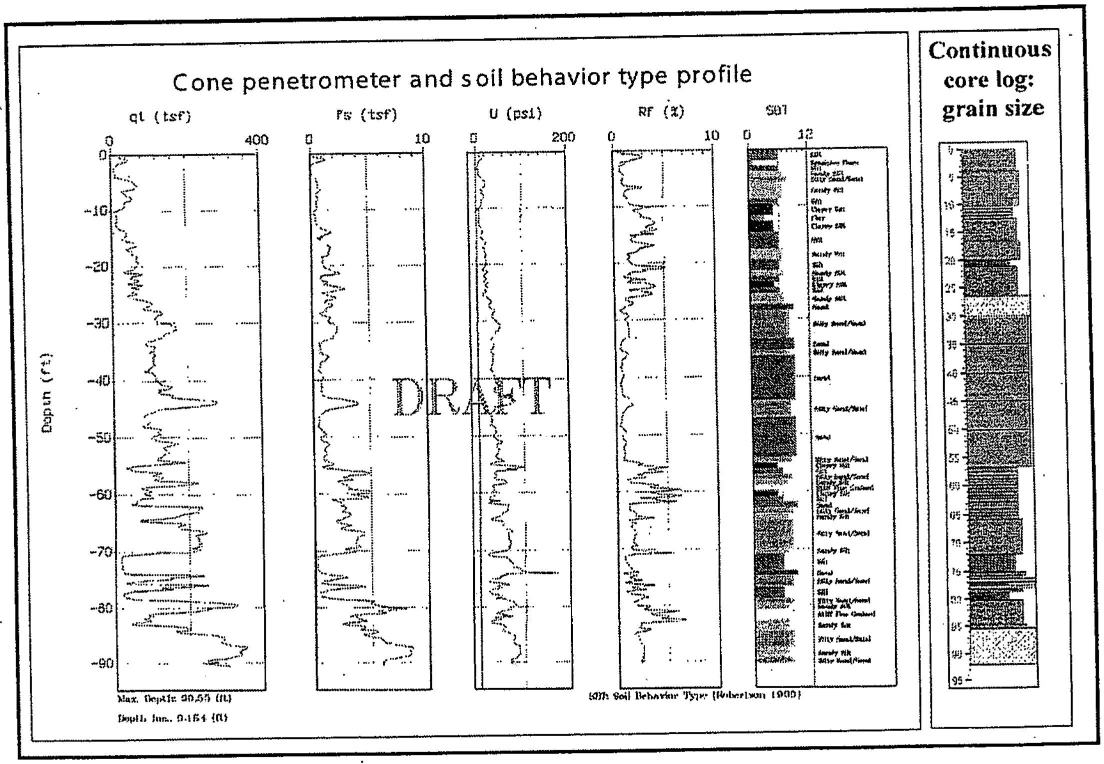

Figure 4. KCD Field Site CPT Logs.
Comparison of soil behavior type (SBT) profile derived from CPT data to sediment texture profile as logged by a State of California certified drilling geologist at the KCD1 Site 1. Depth is shown in feet below ground surface. The thick sequence of sand between 25 and 55 feet shows up in both profiles, as does the confining unit at about 80 feet.

Standard Drilling Methods

Monitor wells were emplaced using standard methods. The first and deepest 200-foot bore-hole was drilled with a mud-rotary rig; subsequent wells were drilled using hollow-stem auger. In the deep 200-foot hole, continuous log core was recovered and logged by a State-certified geologist (Figure 4) and down-hole geophysical data were obtained, including caliper, gamma ray, electro-magnetic induction, and spontaneous potential and resistivity logs. Wells were cased with either 2" or 1.25" PVC pipe with short (generally 2') slotted screens and sand packs, and completed with a sanitary seal. Early wells (installed in 2003) were completed with stovepipe installation, which were subsequently converted to ground-level flush-mount installations in 2004 to accommodate farm activities. All wells installed in 2004 were completed with a flush-mount installation. The 2"-diameter wells were developed using standard bail, surge and pump methods.

Sample Collection and Field Parameters

Groundwater samples were collected after purging the well by either pumping or bailing, after determining water level against a marked datum. Groundwater from production wells was sampled, whenever possible, from upstream of any storage or pressure tank. A variety of methods were used to draw samples from monitor wells, depending on their diameter. Two-inch diameter monitor wells were sampled with a Grundfoss MP-1 submersible pump and Teflon-lined sample line. Smaller 1.25"-diameter monitor wells were sampled with small-diameter Teflon bailers or with a bladder pump and Teflon sample line.

When practical, field measurements of temperature (°C), conductivity (µS/cm), pH, dissolved oxygen (mg/L) and oxidation reduction potential (mV using Ag/AgCl with 3.33 mol/L KCl as the reference electrode) were carried out using a Horiba U-22 ® water quality analyzer. Sampling protocols were specific for different sets of analytes (see sampling sheet in Appendix C), and differed with regard to filtration, sample volume and container, the presence of headspace, and the use of gloves.

Chemical Composition Analysis

Samples for anions and cations were filtered in the field to 0.45 µm, and stored cold and dark until analysis. Anion (NO_3^-, SO_4^{2-}, Cl^-, F^-, Br^-, PO_4^{3-}, NO_2^-) and cation (Ca^{2+}, Mg^{2+}, Na^+, K^+, Li^+, NH_4^+) concentrations were determined by ion chromatography using a Dionex DX-600. Total inorganic and organic carbon (TIC/TOC) was determined on unfiltered samples poisoned with mercuric chloride using a carbon analyzer (OI Analytical TOC Analyzer 1010). Dissolved inorganic carbon (DIC) concentrations were estimated in the water samples by employing the PHREEQC geochemical model (PARKHURST and APPELO, 2002) to achieve charge balance in the samples by adjusting and speciating DIC at the measured pH values. Dissolved organic carbon was also measured in a subset of samples as CO_2 gas pressure after acidification with orthophosphoric acid.

Sediment sulfur and carbon content was determined by elemental analysis by Actlabs (Ancaster, Ontario, Canada). Total C and S were determined on an ELTRA CS 2000 carbon sulfur analyzer. A weighed sample is mixed with iron chips and a tungsten accelerator and is then combusted in an oxygen atmosphere at 1370C. The moisture and dust are removed and the CO_2 gas and SO_2

gas are measured by a solid-state infrared detector. Sulphate S was determined by elemental analysis of the residue from roasting at 850° C. Reduced S was determined by difference. Carbonate C was determined by digestion of the sample in 2 N perchloric acid followed by coulometric titration. Graphitic C was determined by elemental analysis of the residue from roasting at 600° C. Organic C was determined by difference.

Stable Isotope Mass Spectrometry

Samples for nitrate N and O isotopic compositions are filtered in the field to 0.45 µm, and stored cold and dark until analysis. Anion and cation concentrations are determined by ion chromatography using a Dionex DX-600. The nitrogen and oxygen isotopic compositions ($\delta^{15}N$ and $\delta^{18}O$) of nitrate in 26 groundwater samples from KCD1 and MCD were measured at Lawrence Berkeley National Laboratory's Center for Isotope Geochemistry using a version of the denitrifying bacteria procedure (CASCIOTTI et al., 2002) as described in Singleton et al. (SINGLETON et al., 2005). In addition, the nitrate from 34 samples were extracted by ion exchange procedure of (SILVA et al., 2000) and analyzed for $\delta^{15}N$ at the University of Waterloo. Analytical uncertainty is 0.3 ‰ for $\delta^{15}N$ of nitrate and 0.5‰ for $\delta^{18}O$ of nitrate.

Isotopic compositions of hydrogen and oxygen in water ($\delta^{2}H$ and $\delta^{18}O$) were determined at LLNL using a VG Prism II ® isotope ratio mass spectrometer, and are reported in per mil values relative to the Vienna Standard Mean Ocean Water (VSMOW). Isotopic composition of oxygen in water using the CO_2 equilibration method (EPSTEIN and MAYEDA, 1953), and have an analytical uncertainty of 0.1‰. Hydrogen isotope compositions were determined using the Zn reduction method (COLEMAN et al., 1982)

Membrane Inlet Mass Spectrometry (Excess N_2)

Previous studies have used gas chromatography and/or mass spectrometry to measure dissolved N_2 gas (BOHLKE and DENVER, 1995; MCMAHON and BOHLKE, 1996; VOGEL et al., 1981; WILSON et al., 1990; WILSON et al., 1994). Both methods require extraction of a gas sample, which adds time and can limit precision. Membrane inlet mass spectrometry (MIMS) allows precise and fast determination of the concentrations of nitrogen, oxygen and argon dissolved in groundwater samples without a separate extraction step. This method has been used to document denitrification in estuarine and ocean settings (AN et al., 2001; KANA et al., 1994), as well as for detection of volatile organic compounds in water (KETOLA et al., 2002). The MIMS technique has also proven useful for determining excess N_2 from denitrification in groundwater systems (BELLER et al., 2004).

Samples for N_2, O_2, Ar, CO_2 and CH_4 concentration were analyzed by MIMS. A water sample at atmospheric pressure is drawn into the MIMS through a thin silicone rubber tube inside a vacuum manifold. Dissolved gases readily permeate through the tubing into the analysis manifold, and are analyzed using a quadrupole mass spectrometer. Water vapor that permeates through the membrane is frozen in a dry ice cold trap before reaching the quadrupole. The gas abundances are calibrated using water equilibrated with air under known conditions of temperature, altitude and humidity (typically 18 °C, 183 m, and 100% relative humidity). A small isobaric interference from CO_2 at mass 28 (N_2) is corrected based on calibration with CO_2-rich waters with known dissolved N_2, but is negligible for most samples. Typical sample size is 5 mL, and each analysis takes approximately 3 minutes. Dissolved oxygen, methane, carbon dioxide and argon content are measured at the same time as nitrogen. Samples are collected for MIMS analysis in 40 mL amber glass VOA vials, with no headspace, and kept cold during transport. Samples are analyzed within 24 hours to minimize the risk of gas loss or biological fractionation of gas in the sample container. The MIMS is field portable, and can be used on site when fieldwork requires extended time away from the laboratory, or when samples cannot be readily transported to the laboratory.

Noble Gas Mass Spectrometry ($^3H/^3He$ dating)

Dissolved noble gas samples are collected in copper tubes, which are filled without bubbles and sealed with a cold weld in the field. Dissolved noble gas concentrations were measured at LLNL after gas extraction on a vacuum manifold and cryogenic separation of the noble gases. Concentrations of He, Ne, Ar and Xe were measured on a quadrupole mass spectrometer. Calculations of excess air and recharge temperature from Ne and Xe measurements are described in detail in Ekwurzel (2004), using an approach similar to that of Aeschbach-Hertig et al. (2000). The ratio of ^3He to ^4He was measured on a VG5400 mass spectrometer.

Tritium samples are collected in 1 L glass bottles. Tritium was determined by measuring ^3He accumulation after vacuum degassing each sample and allowing three to four weeks accumulation time. After correcting for sources of ^3He not related to ^3H decay (AESCHBACH-HERTIG et al., 1999; EKWURZEL et al., 1994), the measurement of both tritium and its daughter product ^3He allows calculation of the initial tritium present at the time of recharge, and apparent ages can be determined from the following relationship based on the production of tritiogenic helium ($^3He_{trit}$):

$$\text{Groundwater Apparent Age (years)} = -17.8 \times \ln\left(1 + {}^3He_{trit}/{}^3H\right)$$

The reported groundwater age is the mean age of the mixed sample, and furthermore, is only the age of the portion of the water that contains measurable tritium. Average analytical error for the age determinations is ±1 year, and samples with ^3H that is too low for accurate age determination (<1 pCi/L) are reported as >50 years. Loss of ^3He from groundwater is not likely in this setting given the relatively short residence times, lack of water table fluctuations, and high infiltration rates from irrigation. Groundwater age dating has been applied in several studies of basin-wide flow and transport (EKWURZEL et al., 1994; POREDA et al., 1988; SCHLOSSER et al., 1988; SOLOMON et al., 1992). Mean ^3H-^3He apparent ages are determined for water produced from 20 KCD monitor wells at depths of 6 m to 54 m, and from 14 sites at MCD. The apparent ages give a measure of the time elapsed since water entered the saturated zone, but only of tritium-containing portion of the groundwater sample. Apparent ages therefore give the mean residence time of the fraction of recently recharged water in a sample, and are especially useful for comparing relative ages of water from different locations at each site. The absolute mean age of groundwater may be obscured by mixing along flow paths due to heterogeneity in the sediments (WEISSMANN et al., 2002b).

Quantitative Real-Time Polymerase Chain Reaction (rt-qPCR)

We have developed a simple bioassay to quantify populations of denitrifying bacteria in moderate amounts of aquifer material (on the order for a few grams of sediment or filtrate). The method detects the presence of bacterial genes that encode nitrite reductase, a central enzyme involved in denitrification. The assay is not species-specific, but rather a functional test for the presence of bacterial populations capable of nitrite reduction. Nitrite reduction is considered to be the "committed" step in denitrification, and bacteria capable of nitrite reduction are generally also capable of nitric and nitrous oxide reduction to nitrogen gas (TIEDJE, 1988). Currently, the assay provides valuable information on the distribution of denitrifying bacteria populations in aquifers. Ultimately, data on denitrifier populations (i.e., biomass) can be used in combination with specific (i.e., biomass-normalized) denitrification rate constants to determine subsurface denitrification rates.

Real-time, quantitative Polymerase Chain Reaction (rt-qPCR) analysis (Gibson et al., 1996; Heid et al., 1996; Holland et al., 1991), specifically the 5'-nuclease or TaqMan® assay, was chosen for this assay because it offers many advantages over traditional methods used to detect specific bacterial populations in environmental samples, such as DNA: DNA hybridization (Beller et al. 2002). Although most real-time PCR applications to date have involved the detection and quantification of pathogenic bacteria in food or animal tissue, the technique has recently been used to quantify specific bacteria in environmental samples (Hristova et al., 2001; Suzuki et al., 2000; Takai and Horikoshi, 2000).

Real-time qPCR is a rapid, sensitive, and highly specific method. The rt-qPCR assay developed targets two variants of the nitrite reductase gene: *nirS* (Fe-containing nitrite reductase) and *nirK* (Cu-containing nitrite reductase). Homologous gene sequences were used to develop a primer/probe set that encompasses functional *nir* genes of known denitrifying soil bacteria (including heterotrophic and autotrophic species) and that does not result in false positive detection of genes that are not associated with denitrification. The rt-qPCR primers and probes were designed based on multiple alignments of 14 *nirS* and 20 *nirK* gene sequences available in GenBank. During development of the assay, the first nitrite reductase gene (*nirS*) reported in an autotrophic denitrifying bacterium (*T. denitrificans*) was sequenced and amplified, and demonstrated to have high homology to *nirS* in a phylogenetically diverse set of heterotrophic denitrifying bacteria.

Real-time PCR was also be used to quantify total eubacterial population, based on detection of the sequence encoding the eubacterial 16S rRNA subunit, which is specific for bacteria.

Wastewater Co-Contaminants

A number of co-contaminants expected to occur on a dairy farm from the dairy operation proper or from associated field crop production were determined using GC-MS or LC-MS. Co-contaminants targeted included herbicides, pesticides, VOCs, fecal sterols, caffeine and nonylphenol. The analysis of these compounds and a discussion of their distribution at the dairy sites is in Moran et al. (2006).

DATA

Chemical, isotopic, dissolved gas, and groundwater age data for the KCD1 and MCD sites are discussed in Appendix A and Appendix B, and are tabulated in Table 1 of Appendix A and Table 1 of Appendix B. Chemical composition, stable isotope, and groundwater age data for KCD2, KCD3 and SCD2 are tabulated in Table 1 of the main report. In addition, membrane inlet mass spectrometry data for KCD2 is presented graphically in Figures 8 and 9. Neither Appendix A nor Appendix B contains sediment C and S data or bacterial population data, which are discussed below.

Sediment Data

In zones sampled for groundwater at the KCD1 site, sediment texture as determined from well logging, CPT and laser diffraction particle size analysis ranges from sand to clayey silt (with trace to >95% fines). Sedimentary carbonate C is extremely low (generally < 0.003 wt %); organic C is low but generally detectable (0.05-0.10 wt %), although occasional beds have 0.1-1.3% organic C; sulfate S ranges from nondetectable (<0.017) to 0.08 wt%; and reduced S is only detectable in a few wells (<0.01 to 0.15 wt %). For organic C and total S, no strong vertical gradients exist, and no significant difference exists between sediment in the oxic groundwater column, sediment in the anoxic water column, and sediment at the interface. Sediment data are summarized in Table 2, and represented graphically in Figures 5 and 6.

Bacterial Population Data

In this study we use the abundance of the *nir* gene, as determined by rt-qPCR, to map the vertical distribution of denitrifying bacterial populations in the saturated zone. We use the abundance of the eubacterial 16S rRNA gene, as determined by rt-PCR, to map the vertical distribution of total eubacteria in the subsurface. The analyses were performed on soil returned from four locations at the KCD1 dairy during the course of the DP sampling survey in August 2003. Soil samples were placed on ice upon recovery, and subsequently stored frozen until analysis. Total *nir* data are reported as gene copies per 5 g of sediment, and comprise both *nirS* and *nirK* assay results. Total eubacteria data are reported as cells per 5 g sediment. The data are tabulated in Table 3 and in Figure 7.

Relative abundances of *nirS*, *nirK* and eubacteria are consistent with previous studies in non-groundwater systems: *nirS* and *nirK* gene copies typically constitute ~5% and ~0.1% of total bacteria, respectively. Total *nir* abundance varies by almost four orders of magnitude and is not well-correlated with total eubacteria ($R^2 \sim 0.19$ for 5 locations with multiple depths). Peak populations occur either at or below the redoxicline where strong vertical gradients exist in ORP, nitrate and excess nitrogen. Where *nir* abundance is high, total *nir* gene copies tend to constitute a larger fraction of total bacteria (up to 18%).

The presence of high and localized *nir* populations near the interface between oxic high-nitrate groundwater and suboxic low-nitrate groundwater indicates active denitrification is occurring near that interface.

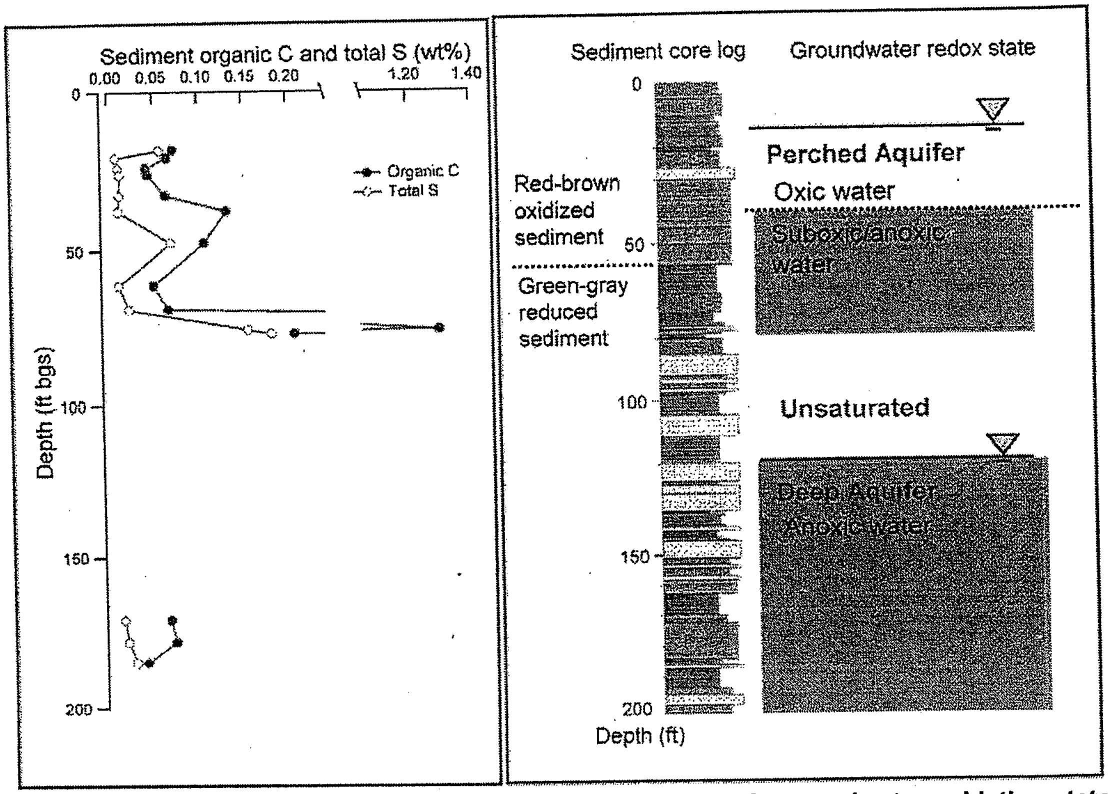

Figure 5. KCD1 Well Cluster 1 sediment composition, texture & groundwater oxidation state
Sediment composition and texture and groundwater oxidation state at KCD1 Site 1. From left to right are shown profiles of sediment organic carbon and total sulfur, sediment iron oxidation state as indicated by sediment color, a continuous core log of sediment texture (yellow sands, brown silty sands, and red silts), the location of the perched and deep aquifer along with groundwater oxidation state (as determined by dissolved oxygen and oxidation-reduction potential probes and the presence of hydrogen sulfide gas).

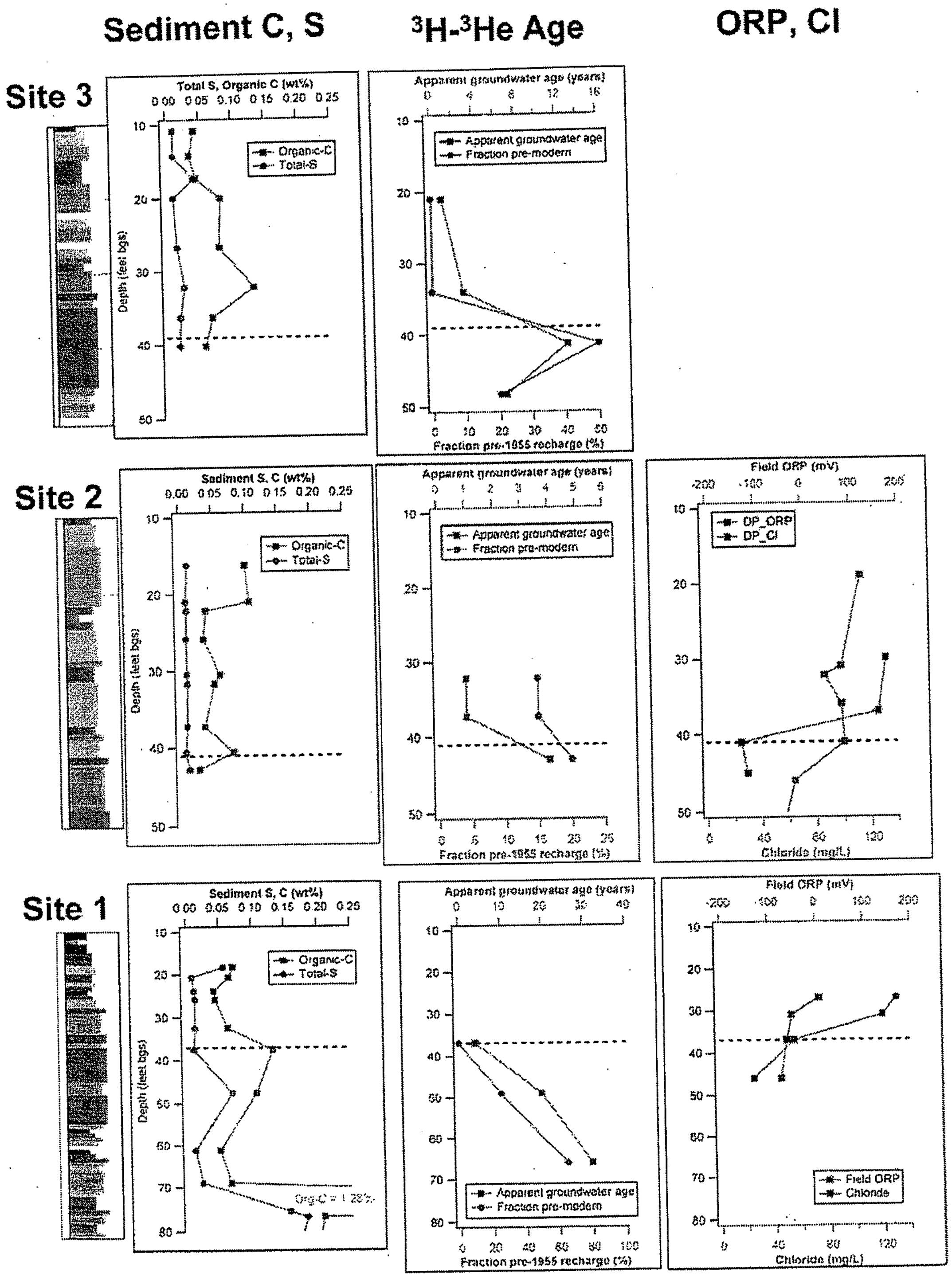

Figure 6. KCD1 depth profiles of sediment and water properties.

KCD1 soil behavior type, sediment organic carbon and total sulfur, ^{3}H-^{3}He groundwater age and fraction pre-modern water, field oxidation-reduction potential (ORP) and dissolved chloride content. The dashed line indicates the transition from nitrate to dissolved nitrogen from denitrification.

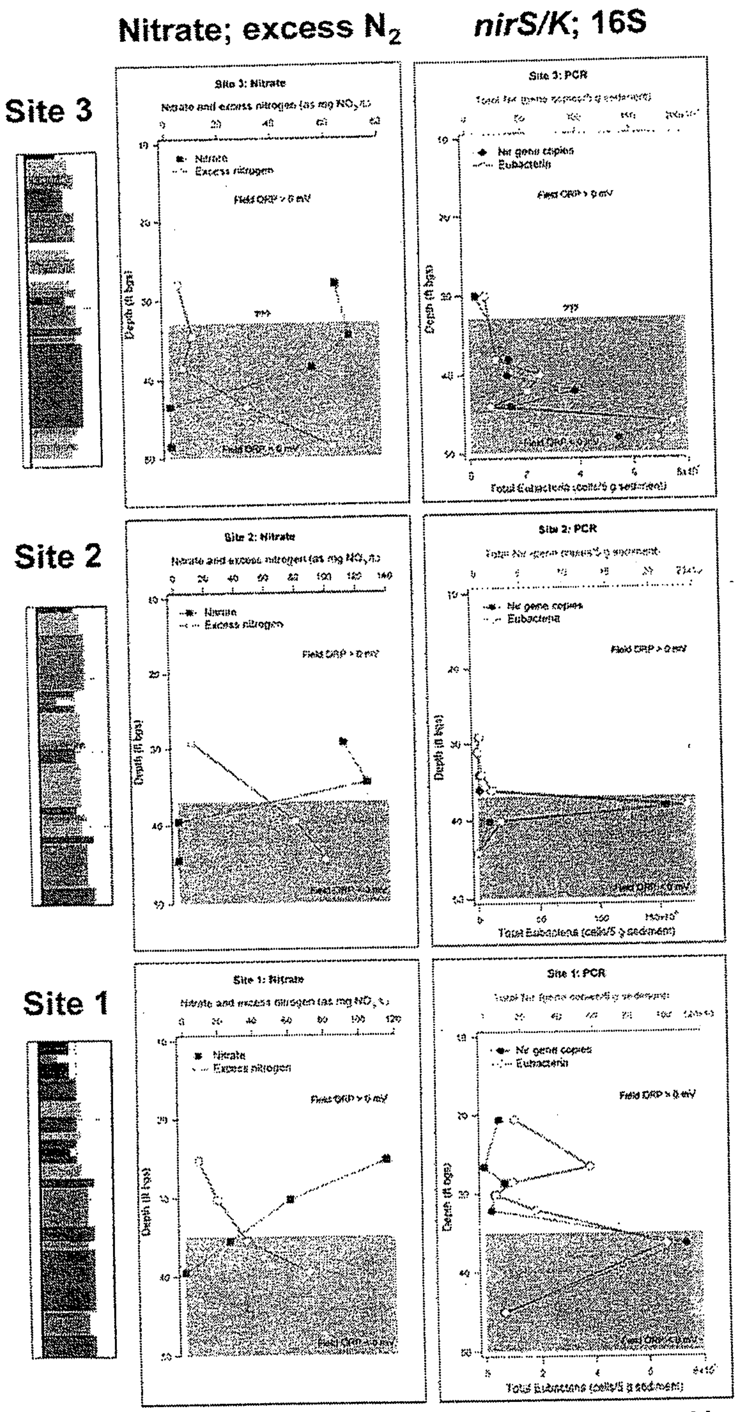

Figure 7. KCD1 depth profiles of nitrogen speciation and bacterial populations.

KCD1 depth profiles of soil behavior type, nitrate, excess nitrogen, total nir gene copies, and total eubacteria. The colored fields indicated water oxidation state based on field ORP.

RESULTS AND DISCUSSION

Saturated-Zone Denitrification at KCD1 and MCD

Appendix A is a manuscript prepared for submittal to a peer-review journal. The manuscript addresses evidence for saturated-zone denitrification in groundwaters impacted by dairy operations. The manuscript abstract follows.

Results from field studies at two central California dairies (KCD1 and MCD) demonstrate the prevalence of saturated-zone denitrification in shallow groundwater with $^3H/^3He$ apparent ages of 30 years or younger. Confined animal feeding operations are suspected to be major contributors of nitrate to groundwater but saturated zone denitrification could effectively mitigate their impact to groundwater quality. Denitrification is identified and quantified using stable isotope compositions of nitrate coupled with measurements of excess N_2 and residual NO_3^-. Nitrate in dairy groundwater from this study has $\delta^{15}N$ values (4.3–61 ‰), and $\delta^{18}O$ values (-4.5–24.5 ‰) that plot with a $\delta^{18}O/\delta^{15}N$ slope of 0.5, consistent with denitrification. Dissolved gas compositions, determined by noble gas mass spectrometry and membrane inlet mass spectrometry, are combined to document denitrification and to determine recharge temperature and excess air content. Dissolved N_2 is found at concentrations well above those expected for equilibrium with air or incorporation of excess air, consistent with reduction of nitrate to N_2. Fractionation factors for oxygen and nitrogen isotopes appear to be smaller ($\varepsilon_N \approx$ -10‰; $\varepsilon_O \approx$ -5‰) at a location where denitrification is found in a laterally extensive anoxic zone 5 m below the water table, compared with a site where denitrification occurs near the water table and is strongly influenced by localized lagoon seepage ($\varepsilon_N \approx$ -50‰; $\varepsilon_O \approx$ -25‰).

Spatial Distribution of Saturated-Zone Denitrification at KCD1

At the KCD1 site, multiple lines of evidence indicate saturated-zone denitrification. These include the presence of excess nitrogen from denitrification at depth, the correlation between nitrate-$\delta^{15}N$ and $-\delta^{18}O$ (which has a slope characteristic of denitrification), and the presence of denitrifying bacteria (which occur at above background levels only where excess nitrogen is present). The lateral extent of denitrification at the site and the excess nitrogen and isotopic evidence for denitrification at the site are discussed in Appendix B. Bacterial distributions give valuable evidence for the localization of denitrification.

Denitrifying bacteria populations at the KCD1 site have a high dynamic range, with peak populations occurring at the oxic-anoxic interface in the perched aquifer where strong gradients in oxidation-reduction potential, nitrate and excess nitrogen exist. Denitrifying bacteria populations are not well correlated with total bacteria ($R^2 \sim 0.19$ for 5 locations with multiple depths). The relative population abundances of *Nir* gene copies, however, are consistent with previous studies in non-groundwater systems: *nirS* and *nirK* gene copies typically constitute ~5% and ~0.1% of total bacteria.

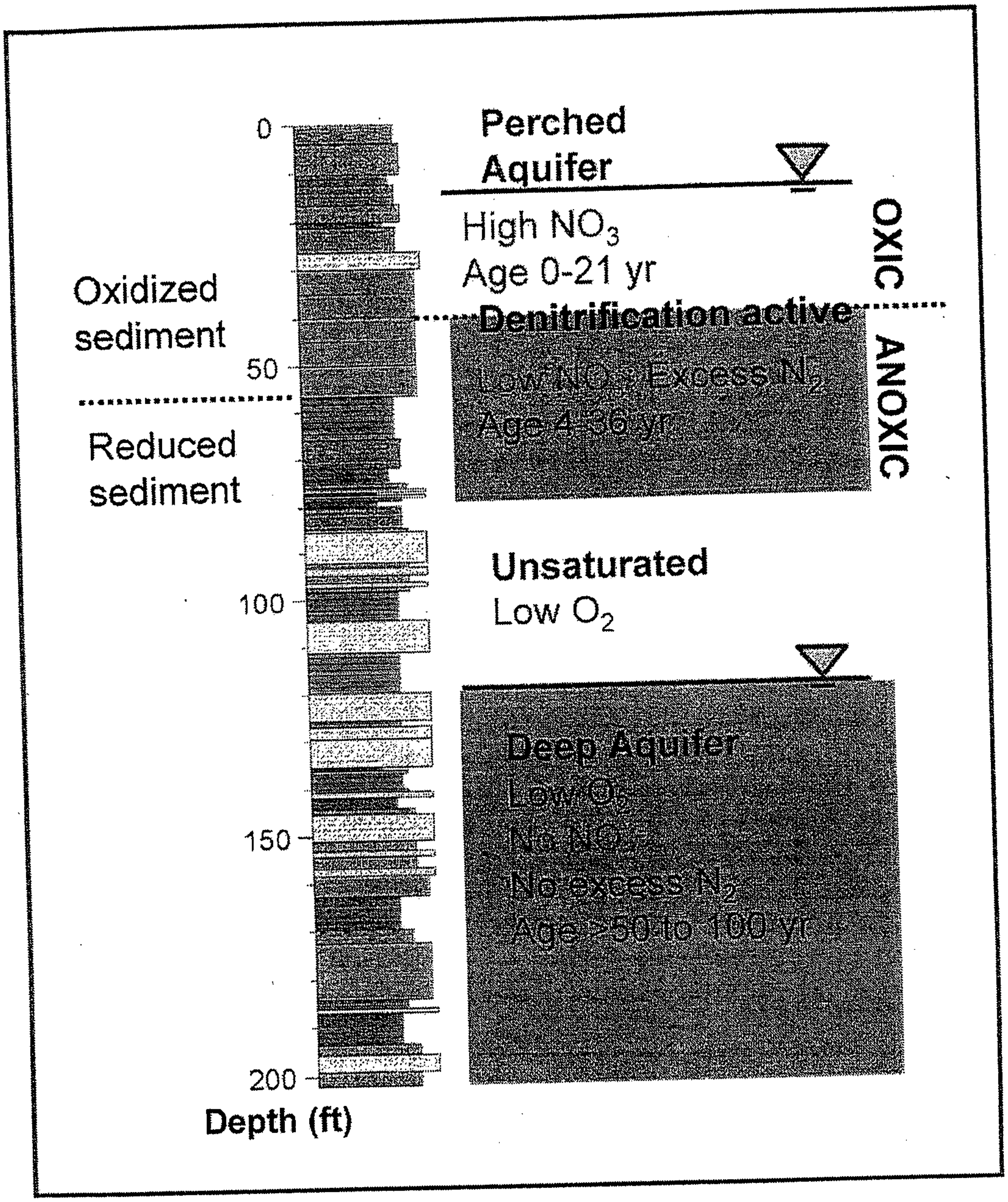

Figure 8. KCD1 site saturated-zone denitrification.

The depth of oxic-anoxic interface is remarkably constant at 37-41 feet below ground surface (Figure 7). This transition is not strongly correlated with lithology or sediment composition (organic-C or total-S content), although it generally occurs in sand. At the irrigated field monitoring sites, the redox interface corresponds to the interface between shallower "young" groundwater (having young apparent ^3H-^3He ages and low mixing ratios of pre-1955 water) and deeper "old" groundwater (with higher fractions of pre-modern water) (Figure 8). The depth of the zone corresponds to the top of several agricultural production pump screens in the area, suggesting that pumping may be a factor.

Saturated-Zone Denitrification at the Northern Dairy Sites

Both of the northern San Joaquin Valley dairy sites (MCD and SCD) are a part of the northern San Joaquin Valley monitoring network described in Harter et al. (2002). Chemical data from these sites have been used to calibrate and validate regional models for nitrogen loading to the shallow groundwater system (VAN DER SCHANS, 2001). The wells sampled are all shallow piezometers that draw first-encounter water, with the exception of one deeper domestic supply well (W-98, Table 1 of Appendix A). A significant finding of the current study is that evidence for saturated-zone denitrification at MCD and SCD only exists in first-encounter wells that are predicted by other criteria (groundwater gradient, the presence of ammonia, total dissolved solids, etc) to be impacted by recharge from lagoons or corrals, i.e. from the dairy operation proper. Wells so impacted include W02, W03, W16, W17, V01, and V21 on the MCD site (Table 1 of Appendix A), and Y03 and Y10 on the SCD site (Table 1). No evidence for denitrification exists in first-encounter wells that are impacted only by wastewater irrigation of either field crops (MCD) or of orchards (SCD). This finding is significant in two respects:

- The UC-Davis nitrate loading model for the region is in agreement with available spatial and time-series groundwater nitrate concentration data. The model does not explicitly consider denitrification of nitrogen fluxes from lagoons and corrals. The absence of evidence for denitrification in first encounter groundwater impacted by wastewater irrigation validates the model assumption that denitrification is not occurring and strengthens confidence in the model as a predictive tool.

- The deep domestic well W-98 is predicted by the UC-Davis model to have approximately 50 mg/L nitrate (T. Harter, personal communication). Groundwater from this well actually has very low nitrate (0.4 mg/L), but does have 45 mg/L nitrate-equivalent of excess N_2 indicating that the mass fluxes and transport in the model are accurate. The mean $^3He/^3H$ groundwater age also matches well with model travel time predictions. The good agreement between predicted nitrate and excess nitrogen in W-98 is consistent with a groundwater impacted by wastewater irrigation in which denitrification is occurring at some depth below the water table, as is the case at KCD1 in Kings County.

- The association of denitrification with groundwater impacted by manure lagoon seepage is consistent with the findings from the KCD1 study (see Appendix B)

To the extent that saturated-zone denitrification is significant and is associated with nitrogen loading from wastewater irrigation from dairy operations (as has been shown on one site, and indicated on another), the process needs to considered when assessing total impact of dairy operations on the groundwater resource. The most effective way to characterize saturated-zone denitrification is the installation of multi-level monitor wells in conjunction with the determination of nitrate stable isotope composition and excess nitrogen content.

The Impact of Dairy Manure Lagoons on Groundwater Quality

Appendix B is a manuscript prepared for submittal to a peer-review journal. The manuscript addresses the impact of dairy manure lagoon seepage on groundwater quality, and discusses a new tracer for manure lagoon seepage. The manuscript abstract follows.

Dairy facilities and similar confined animal operation settings pose a significant nitrate contamination threat to groundwater via oxidation of animal wastes and subsequent transport through the subsurface. While nitrate contamination resulting from application of animal manure as fertilizer to fields is well recognized, the impact of manure lagoon leakage on groundwater quality is less well characterized. For this study, a dairy facility located in the southern San Joaquin Valley of California (KCD1) has been instrumented with monitoring wells as part of a two-year multidisciplinary study to evaluate nitrate loading and denitrification associated with facility operations. Among the multiple types of data collected from the site, groundwater and surface water samples have been analyzed for major cations, anions, pH, oxidation-reduction potential, dissolved organic carbon, and selected dissolved gases (CO_2, CH_4, N_2, Ar, Ne). Modeling of geochemical processes occurring within the dairy site manure lagoons suggests substantial off-gassing of CO_2 and CH_4 in response to mineralization of organic matter. Evidence for gas ebullition is evident in low Ar and Ne concentrations in lagoon waters and in groundwaters downgradient of the lagoon, presumably as a result of gas "stripping". Shallow groundwaters with Ar and Ne contents less than saturation with respect to atmosphere are extremely rare, making the fractionated dissolved gas signature an effective tracer for lagoon water in underlying shallow groundwater. Preliminary evidence suggests that lagoon water rapidly re-equilibrates with the atmosphere during furrow irrigation, allowing this tracer to also distinguish between seepage and irrigation as the source of lagoon water in underlying groundwater. Together with ion exchange and mineral equilibration reactions, identification of lagoon seepage helps to constrain key attributes of the local groundwater chemistry, including input and cycling of nitrogen, across the site.

A New Tracer for Manure Lagoon Seepage

The manuscript in Appendix B uses only data collected from the KCD1 site. We also see evidence for gas stripping in lagoon waters from the KCD2 site (Figure 9). To further test the hypothesis that gas stripping in biologically active manure lagoons, we sampled manure lagoon water from several locations at KCD2 site. At this site, manure-laden water flows from free stall flush lanes to a settling lagoon (Lagoon 1) through an intake near the bottom of the lagoon to a larger holding lagoon (Lagoon 2) to a distribution standpipe to furrows in nearby fields. Samples were collected from the surface of Lagoon 1 near the outtake from the flush lanes, from the outlet of Lagoon 1 into Lagoon 2, from the surface of Lagoon 2 near the intake to the field distribution system, from a distribution standpipe, and from a field furrow about halfway down the length of the furrow. At the time of sample collection in April 2005, water in the distribution standpipe and in the field furrows was entirely from the manure lagoon, and was not mixed with well water or canal water. The results are shown in Figure 10.

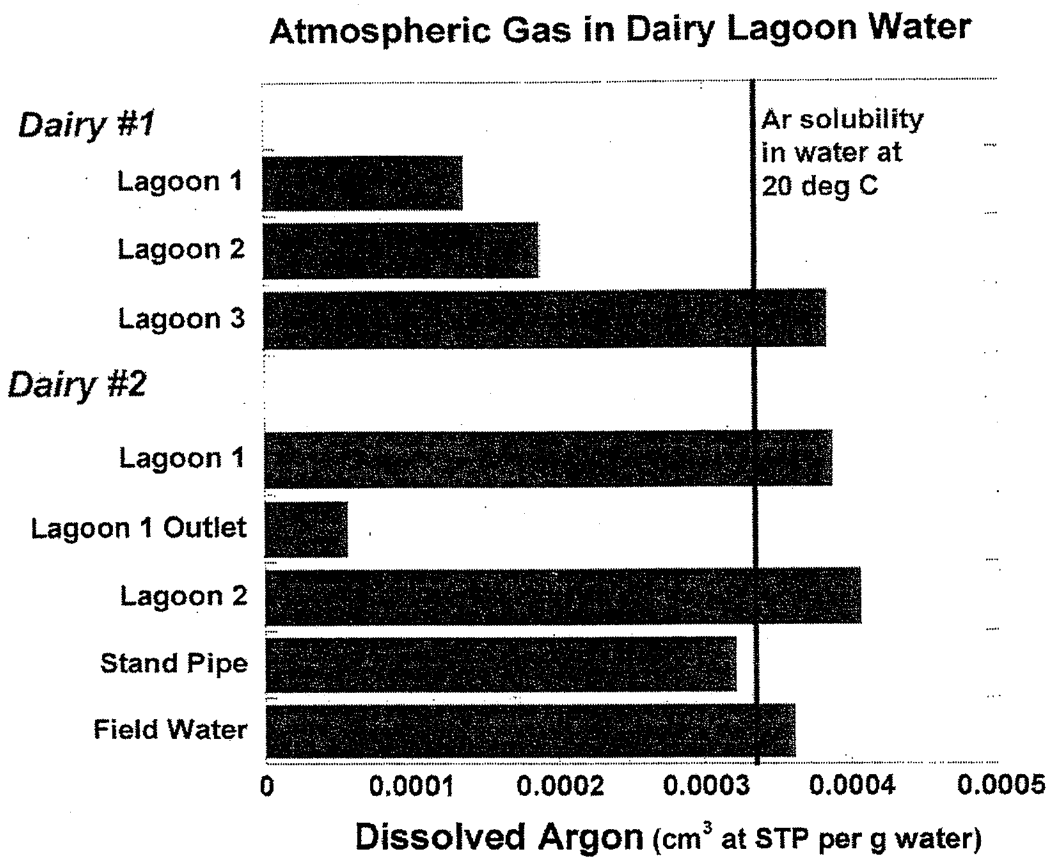

Figure 9. KCD1 and KCD2 manure lagoon dissolved argon content.

As discussed in Appendix B, biological activity in the lagoon consumes oxygen and strips atmospheric gases from the lagoon water through ebullition of carbon dioxide and methane. This effect of this activity is evident in the absence of detectable oxygen in any of the lagoon samples, and in lagoon water argon partial pressures that are close to or far below saturation argon partial pressures. For non-reactive gases such as argon, the "gas-stripping" effect is most evident in the sample drawn from the outlet of Lagoon 1 into Lagoon 2, which presumably represents water from near the bottom of Lagoon 1. This sample has extremely low argon, and may be representative of lagoon seepage through the bottom or sides of the lagoon. Atmospheric re-equilibration does not take place until the water is delivered to the field – the water sample drawn from the distribution standpipe has no detectable oxygen, while surface water from half-down a furrow is at about 40% saturation. We suspect that percolation through the soil zone and through an oxic vadose zone, which is characterized by incorporation of excess air, will result in complete re-equilibration or over-equilibration with soil gases.

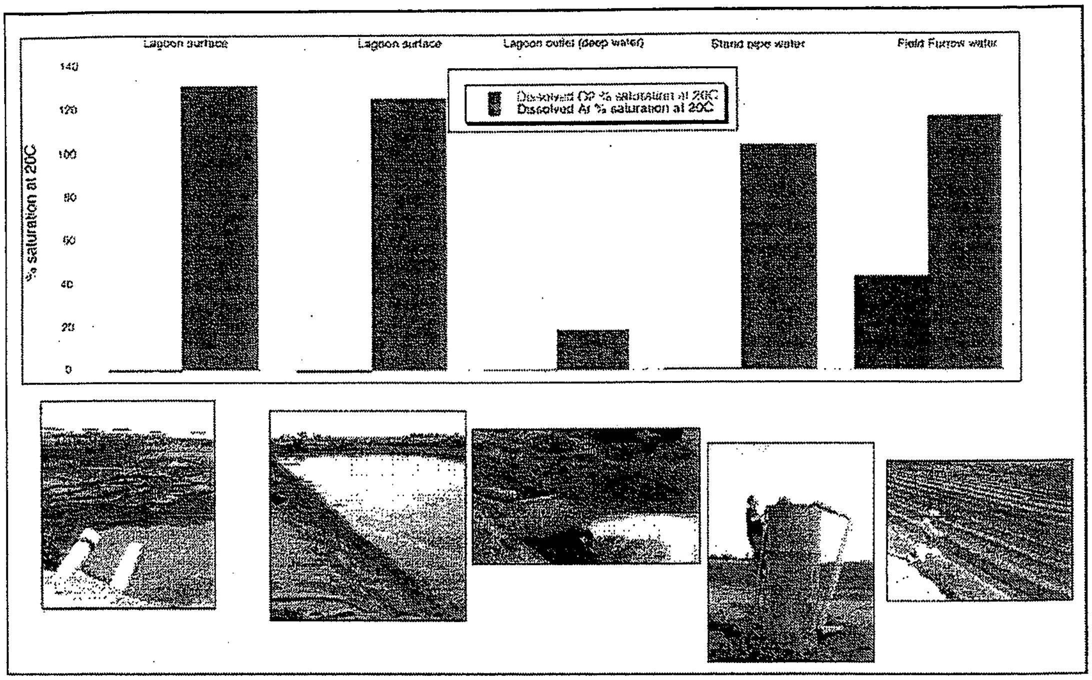

Figure 10. Dissolved argon and oxygen at KCD2.

The evolution of dissolved argon and dissolved oxygen along a "flow path" at KCD2. From left to right in figure: Lagoon 1 surface water , Lagoon 2 surface water, Lagoon 1 outlet into Lagoon 2, an irrigation standpipe, and a field furrow. Note that the Lagoon 1 outlet precedes the Lagoon 2 surface water in the "flow path". See text for explanation.

Dissolved gas samples from a number of manure lagoons on five dairy sites (KCD1, KCD2, MCD, and SCD) are characterized in general by deficiency in reactive and non-reactive atmospheric gases, and in detail by a wide range in non-reactive gas pressures from near equilibrium to far below equilibrium. The only other mechanism known to produce such signals is methane production either in marine sediments or in the deep subsurface in association with natural gas formation (see references in Appendix B). Currently the presence of an air "deficit" (i.e. atmospheric noble gases below saturation values) in shallow groundwater samples associated with dairy operations can be considered as indicative of the presence of a manure lagoon seepage component. To determine the mixing ratio of lagoon seepage with other water sources, however, will require a more quantitative understanding on the dissolved gas content in manure lagoons and manure lagoon seepage.

Source, Fate and Transport of Dairy Nitrate at KCD1

Harter et al. (2002) have demonstrated that dairy operations in the northern San Joaquin Valley strongly impact groundwater quality, resulting in first-encounter water that is high in salinity and inorganic nitrogen. On the KCD1 site in the southern San Joaquin Valley, a number of observations indicate that the dairy operation and associated wastewater irrigation are the source of high nitrate in first encounter groundwaters at the site:

- The isotopic composition of nitrate-N and –O is consistent with a manure or septic nitrogen source (see Appendix A).

- The young age of the first encounter waters (Figure 6 and 8), which we have accurately simulated using an irrigation recharge model (see groundwater transport discussion below) are inconsistent with transport from offsite locations.

- Nitrate co-contaminants can be traced to a specific application event on the site (see MORAN, 2006). In a subset of wells on the site, norflurazon and its degradation product, desmethylnorflurazon, were detected. Norflurazon was applied to a corn field in excess of the intended amount approximately two years prior to sampling. The well closest to the field contains norflurazon; a more distal well contains the degradation product, desmethylnorflurazon.

The unconfined aquifer at KCD1 is strongly stratified with respect to electron donor concentration (oxygen and nitrate), redox state (ORP), and excess nitrogen (Figures 5 and 6). The transition zone is sharp: nitrate levels can drop from significantly above maximum contaminant levels to nondetectable over a depth range of five feet. Our data indicate that the water immediately below the transition zone also has a significant wastewater component:

- Low-nitrate groundwaters nitrate isotopic compositions that are consistent with denitrification of manure or septic source nitrate.

- Some low-nitrate waters have below-saturation dissolved gas pressures that indicate a component of manure lagoon seepage (see Appendix B and discussion below.)

- Groundwater transport modeling (see discussion below) that assumes recharge dominated by wastewater irrigation accurately simulates the mean age and pre-modern mixing rations for low-nitrate groundwaters below the transition zone.

The strong spatial association of high denitrifier bacterial populations (Figure 6) with the transition zone is consistent with active denitrification occurring in this zone and being at least one source of denitrified groundwater seen below the zone. We cannot currently convert *nir* gene copy populations into denitrification rates, and so cannot estimate what fraction of denitrification occurs in the transition zone and what fraction occurs upgradient (proximal to a manure lagoon seepage plume, for example). What is clear, however, is that active denitrification is currently occurring on the dairy site in localized subsurface zones.

The relationship of the dairy operation (including wastewater irrigation and manure lagoon seepage) to nitrate mitigation through the establishment of redox stratification and the enhancement of saturated-zone denitrification is more complex. Any model of the evolution of redox stratification and denitrification must first provide an electron donor and then produce a sharp transition zone (~5 feet in vertical extent) at a remarkably uniform depth across the site (~35-40 feet bgs). A number of hypotheses can be put forward:

- Lateral transport of manure lagoon seepage.

- Field irrigation with dairy wastewater (assuming vertical percolation through a homogeneous soil column that contains a solid-phase electron donor).
- Agricultural pumping and nitrogen loading from dairy operations (assuming strong lateral transport of nitrate through a heterogeneous aquifer).

The Impact of Lagoon Seepage on Groundwater Quality

The first hypothesis is discussed in McNab et al. (Appendix B and Figure 11).

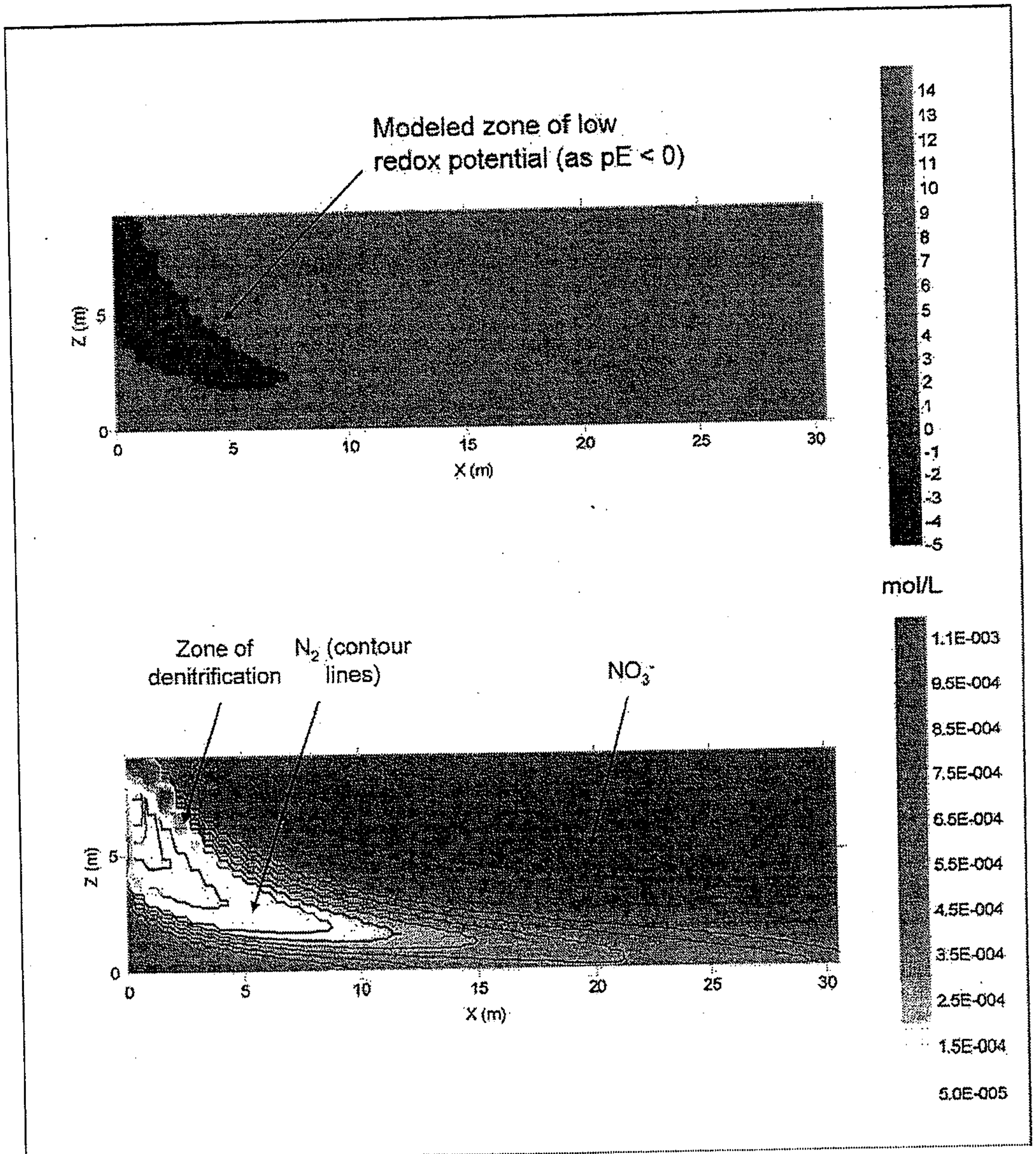

Figure 11. Simulation of transport of lagoon seepage through groundwater.
Simulation of the influence of seepage from a dairy wastewater lagoon on groundwater chemistry. See Appendix B for details on modeling.

McNab et al. assume that oxidation of organic carbon derived from manure creates the reducing conditions and provides the electron donor necessary for denitrification. While manure lagoon seepage is associated with excess nitrogen and does appears to drive denitrification locally, reactive transport modeling of lagoon seepage shows that the modeled zone of denitrification does not extend far from the lagoon, and that the modeled zone of low redox potential (where pE < 0) is localized (Figure 11). These model results are driven by the relative magnitudes of lagoon seepage and wastewater irrigation percolation rates, and are consistent with dissolved gas evidence indicating that lagoon seepage is not a major component in most site groundwaters. We conclude that manure lagoon seepage is not the cause of the laterally extensive reduced zone observed at the KCD1 site.

The Impact of Dairy Wastewater Irrigation on Groundwater Quality

Reactive transport modeling of vertical flow under an irrigated field indicates that vertical redox stratification can be created without a lagoon influence when dairy wastewater percolates through a soil column containing organic carbon in low permeability micro-environments. Attempts to simulate the development of redox stratification in the absence of a sedimentary electron donor were not successful.

We employed a reactive modeling approach using PHREEQC that addresses multispecies solute transport, soil-water reactions (mineral phase equilibria and ion exchange), and reaction kinetics for redox reactions involving nitrogen species as means for identifying the potential roles of different electron donors in the denitrification process at the site. The model parameters are shown below:

Parameters

- 10-m column
    - 10 volume elements (mobile pore water)
    - 10 volume elements (immobile pore water)
- Initial sediment composition:
    - 25% Quartz
    - 15% Na-montmorillonite (ion exchanger)
    - 15% K-mica ("C" model; no K-mica = "X" model)
    - 1% Goethite (HFO surface)
    - 0.02 mol/kg organic carbon

Step 1: Set up initial conditions

- Flush column with 300 pore volumes:
    - 1 mM NaCl
    - mM KCl
- After flushing
    - Equilibrium with CO_2(g) and O_2(g), calcite, and dolomite
    - Undersaturated with gypsum

Step 2: Simulate irrigation

- Flush column with 2 pore volumes with a mixture of agricultural well water and lagoon water (~0.02 M NH4+; ~0.01 M K+) – agricultural well water.
- Allow equilibration with calcite, ion exchanger, and HFO surface.

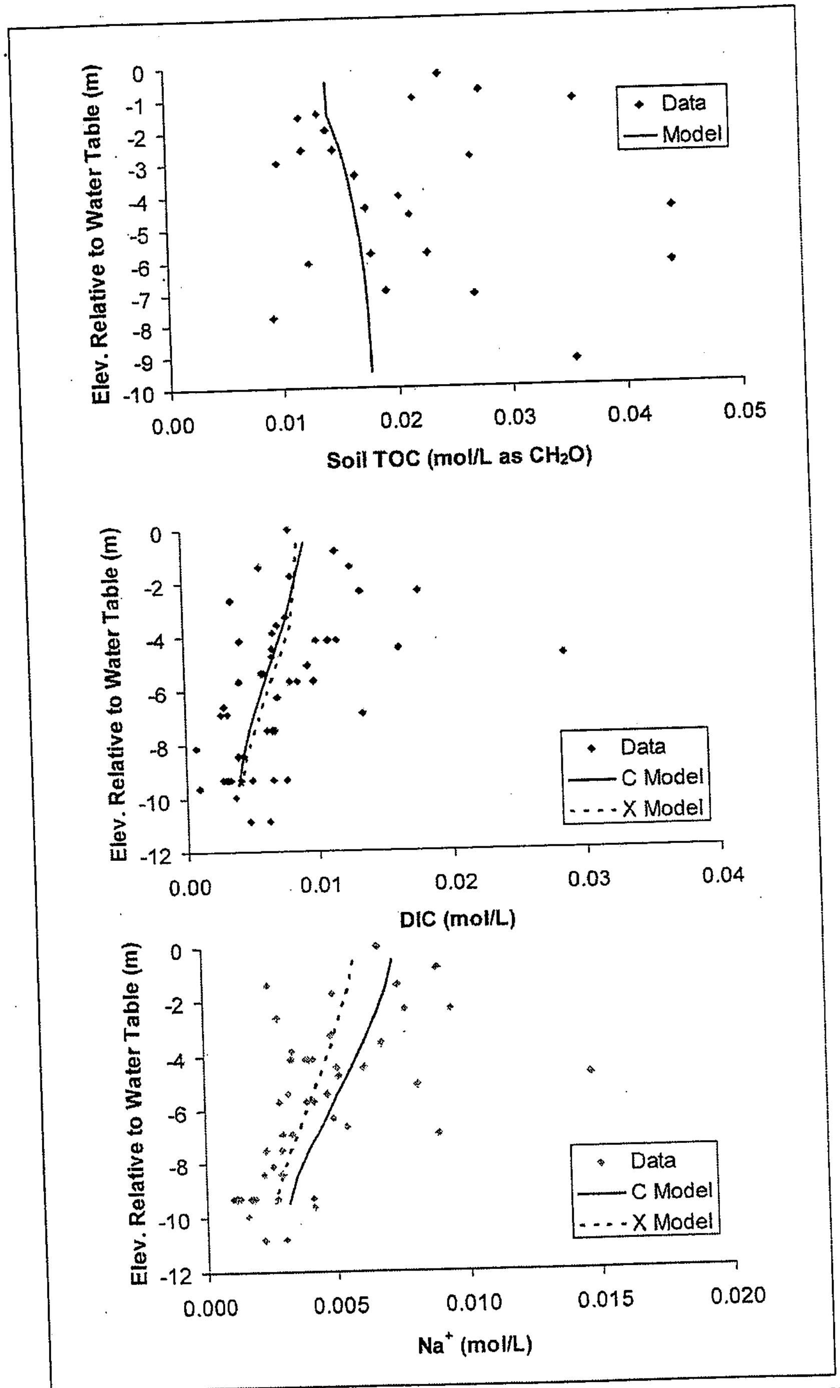

Figure 12. Simulation of dairy wastewater percolation through sediment.
Model results from simulation of vertical percolation of dairy wastewater through a sediment column containing organic carbon in low-permeability environments. See text for explanation.

Results from the reactive transport simulations results generally match most major cation and anion distributions with depth (Figure 12 and Figure 13). Moreover, the quantities of organic carbon required to produce a redox front (via diffusion-limited transport through low-permeability lenses) are consistent with measurements from soil samples (which are low). These results do not depend on any lagoon influence. Reactive transport modeling of vertical flow under the irrigated field demonstrates that general geochemistry in wells distal from the manure lagoons can be explained *without* postulating a lagoon influence, if the aquifer has reducing capacity.

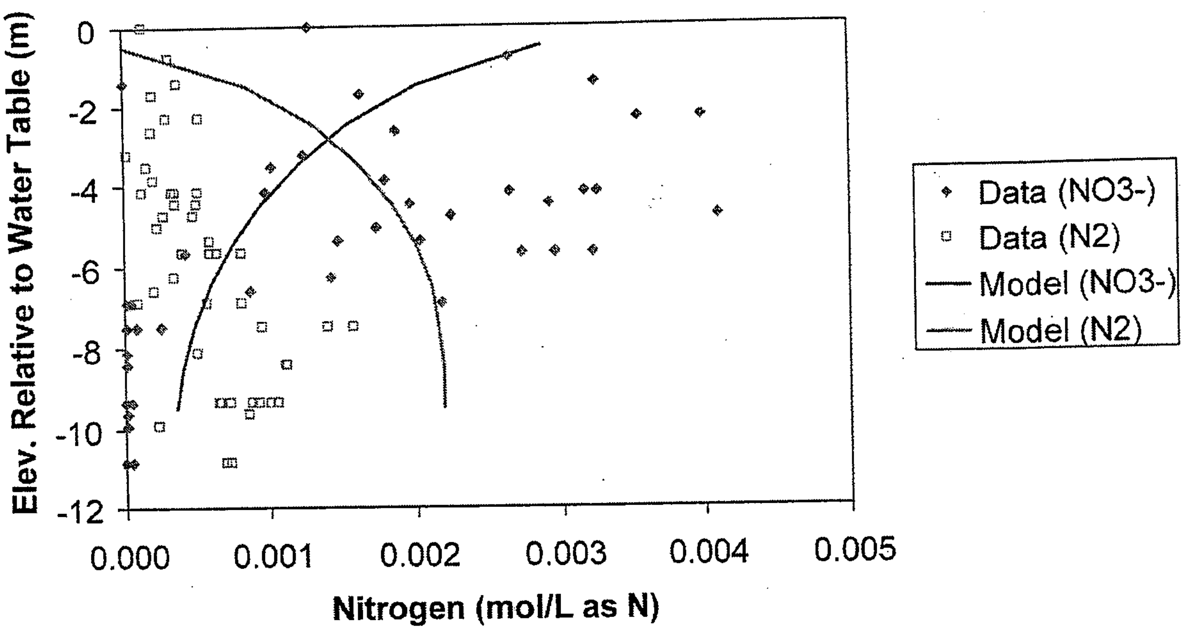

Figure 13. Simulation of denitrification associated with dairy wastewater percolation.
Saturated-zone denitrification in a simulation of vertical percolation of dairy wastewater through a sediment column containing organic carbon in low-permeability environments. See text for explanation.

A number of lines of evidence exist that indicate that reducing groundwater conditions are common in the region surrounding the KCD1 site. At a number of NAWQA sites in the region that are not believed to be impacted by dairy wastewater, nitrate in deeper waters is nondetectable and iron and manganese concentrations are high, an association consistent with suboxic or anoxic conditions (BUROW et al., 1998a; BUROW et al., 1998b). The most convincing evidence comes from the deep well at the KCD1 site (KCD1-1D, Table 1 in Appendix A). Groundwater in the lower aquifer sampled by this well is tritium dead with a mean groundwater age in excess of 50 years. Radiogenic ^{4}He content indicates an age on the order of 100 years or more. Neither nitrate nor excess nitrogen is present, indicating that source waters were low in inorganic nitrogen species. This groundwater has extremely low chloride and has isotopically lighter water than water sampled in the perched aquifer. Finally, this groundwater is reduced as indicated by both field ORP and DO measurements, and measurements of volatile sulfide compounds in the water. These observations are consistent with recharge by source waters unimpacted by agriculture and the occurrence of naturally reducing conditions along the flow path. The electron donor driving the evolution of the natural reducing system is unclear. The water is low in TOC (0.8 mg/L). Sediment organic C and reduced S contents are generally low (< 0.1 wt %), but are sufficient to produce reducing conditions, particularly since sediments with organic carbon contents of over 1 wt% have been characterized (Figures 5 and 6). Reducing conditions may have also been created during recharge (in the hyporheic zone during riverbank infiltration).

The existence of regionally reducing conditions is also evident in the redox state of sedimentary iron in site sediments. Above approximately 60' bgs, sediment core is stained with orange, red and brown ferric iron oxides; below 60', this stain is not present (Figures 5 and 8). The existence of a denitrification zone approximately 20-25' above the iron reduction zone is consistent with the energetics of these reactions.

Given the presence of reducing conditions within the aquifer, one-dimensional transport through homogeneous media can drive the development of redox stratification and saturated-zone denitrification within the shallow aquifer. This process, however, can only reproduce the sharpness and uniform depth of the observed groundwater redox stratification 1) if a layer of laterally extensive reducing sediment exists at the groundwater redox boundary or 2) if a sharp transition in sediment reducing capacity exists at or near the depth of the water redox transition. Neither of these conditions is observed at the KCD1 site. The redox boundary is not correlated with sediment texture, nor do any gradients exist in sedimentary organic C, total S, or reduced S that correlate with the depth of the redox boundary.

The Impact of Pumping and Wastewater Irrigation on Groundwater Quality

A number of processes that may contribute to strong vertical stratification of groundwater flow and chemistry are not adequately simulated in a one-dimensional homogeneous model. To explore the effect of aquifer heterogeneity and lateral transport on groundwater flow and transport at the KCD1 site, we used the numerical flow and transport model NUFT to simultaneously simulate three-dimensional variably-saturated groundwater flow processes including canal recharge, agricultural pumping, and irrigation (CARLE et al., 2005). Heterogeneity of sandy, silty, and clayey zones in the system was characterized stochastically by applying transition probability geostatistics to data from 12 CPT logs that vertically transect the perched aquifer. In the first iteration of this model, nitrate in surface irrigation was simulated as a tracer rather than as a reactive species.

Groundwater Hydrology. In the distal reaches of the Kings River within the Tulare Lake Basin, groundwater is extracted from both a perched zone (less than ~ 25 m deep) and a deep zone. Before the 1950's, water levels were nearly equal in both zones (DWR data). Overdraft in the deep zone has caused water level declines of over 100 feet (30 m). Perched zone water level elevations, where they exist, persist well above the deep zone, as evident from DWR water level elevation maps for 2001-2002. The Kings River, unlined ditches and canals, and irrigation appear to provide recharge to sustain the perched aquifer. Crop irrigation uses canal diversions and both shallow and deep groundwater.

At and near the KCD1 site, groundwater level elevations in different wells screened in the perched aquifer are remarkably similar over time and correlate to canal diversions. This suggests canal leakage and irrigation from canal diversions provides substantial recharge to the perched aquifer. Leakage from the canal is estimated at 10% by the irrigation district.

Several dairies are located within the area of the perched aquifer. KCD1 is located about one mile east of the canal. The dairy grows much of its own feed – corn and alfalfa. The crops are irrigated primarily with water pumped from the shallow aquifer. Crops are fertilized largely by mixing in effluent from the dairy operation that is collected in a lagoon. The lagoon water and other fertilizers provide sources of nitrate that appear to impact upper portions of the perched aquifer, but not lower portions of the perched aquifer or the deep aquifer. Other nearby farms also irrigate with canal diversions or groundwater pumped from the deep aquifer. Thus, overdraft from the deep aquifer helps, in part, to sustain the perched aquifer.

The modeling approach was designed to include consideration of the major factors and processes affecting groundwater flow, nitrate transport, and groundwater age dating:

- *Heterogeneity*: Use hydrofacies-based geostatistics.
- *Variably Saturated Flow:* Couple vadose zone and saturated zone using LLNL's NUFT code.
- *Boundary Head Conditions:* Use time-series DWR water levels in perched and deep zone.
- *Perched and Deep Zone:* Use modeling to determine leakage that maintains perched condition.
- *Canal Leakage and Irrigation:* Distinguish different sources with different tracer simulations.
- *Tritium/Helium-3 Age Dating:* Add decay to tracer simulations, simulate apparent age estimate.
- *Groundwater Mixing:* Keep track of proportions of groundwater from different sources.

Heterogeneity. Based on our interpretation of lithologic and CPT logs, we defined three hydrofacies: "sand", "silt", and "clayey" categories. We quantified vertical and horizontal spatial variability with a transition probability matrix using the CPT data categorized as hydrofacies. The solid lines in the probability matrices (Figure 14) represent 1-D Markov chain models used to develop stochastic simulations of hydrofacies architecture at the site.

The hydraulic properties of the hydrofacies categories were estimated from a combination of pump test analysis, soil core measurements, and model calibration.

HYDROFACIES	K (m/d)	POROSITY
Sand	30	0.40
Silt	0.24	0.43
Clayey	0.014	0.45
Sandy Loam Soil	3.0	0.41
Aquitard	1.4e-6	0.45
Canal (sandy)	10.0	0.41

A Van Genuchten model was used to predict unsaturated hydraulic conductivity and capillary pressure. A continuous 1-m thick aquitard layer at 46-47 m elevation sustains the perched aquifer conditions. This aquitard layer correlates to a distinctive clay layer identified in our initial characterization lithologic log.

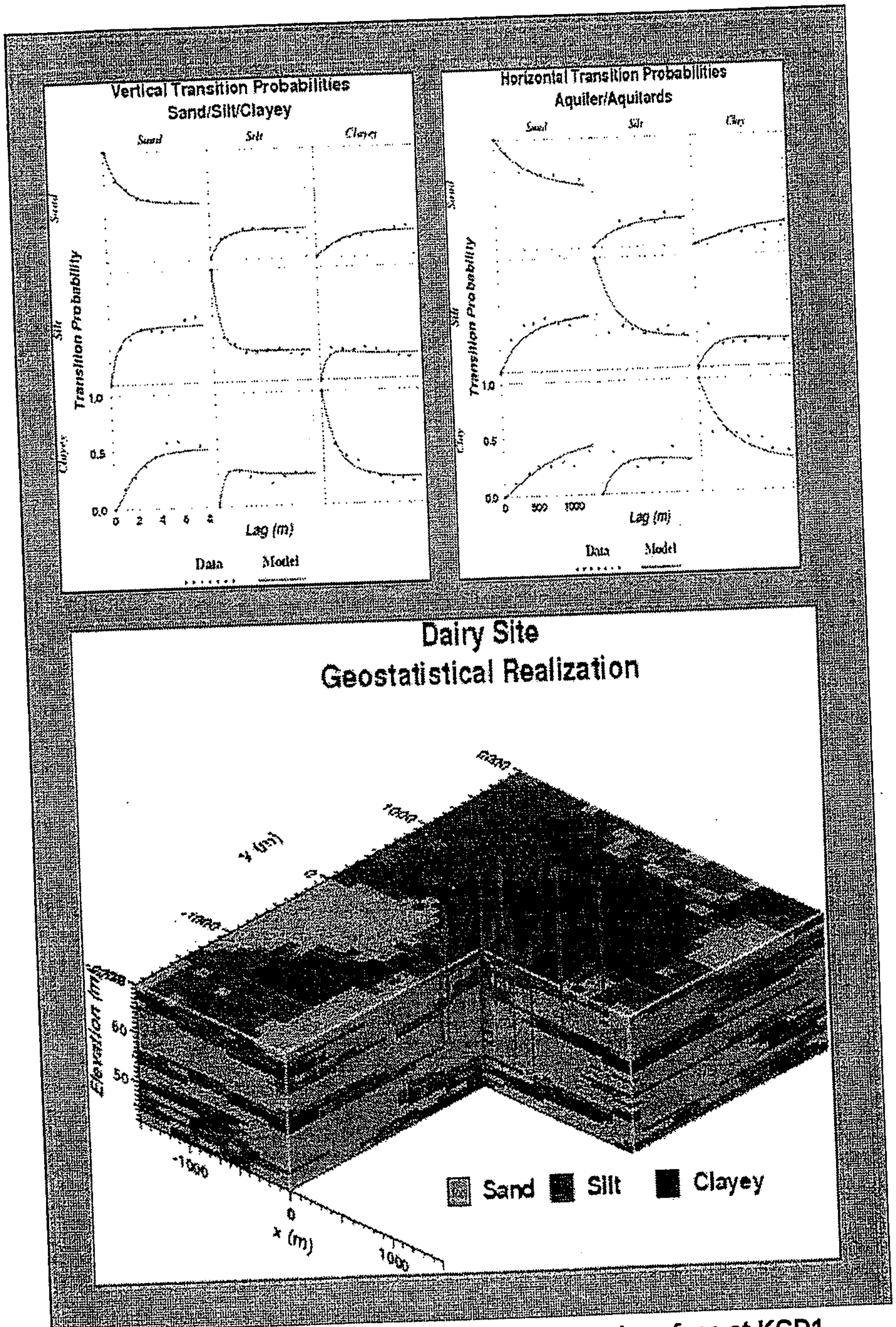

Figure 14. Geostatistical representation of the subsurface at KCD1.
Transition probability matrices and geostatistical representation of hydrofacies architecture for the KCD1 site. See text for explanation.

Flow and transport simulation (Figure 15 and 16). We used LLNL's NUFT code to simulate variably saturated **flow** according to the Richards equation (Figure 15). The simulation runs from late 1949 through 2001. Initial conditions are equilibrated to local head measurements and rainfall recharge of 1 cm/year. For boundary conditions, x-direction and bottom boundaries were conditioned to observed piezometric heads. A fully saturated initial condition is applied to the canal when canal diversions occur (between early April and early October). In the simulation, the six site production wells were pumped during irrigation season a rate greater and proportionate to crop evapotranspiration (ET). Recharge from irrigation was distributed proportionately to crop (ET), with about 25 cm/yr within the dairy crop fields and 10 cm/yr in surrounding areas.

In the simulation, piezometric head in the perched aquifer remains relatively steady, although in fall 1992 (during a drought) head is noticeably lower. However, head in the deep aquifer drops considerably since the 1950s, to the extent that the top of the deep zone begins to desaturate in the 1960s. In effect, the aquifer system near the dairy field site now functions like two unconfined aquifers stacked on top of each other. This is consistent with the observed separation of the DWR water levels between shallow and deep wells in the 1960s.

We used LLNL's NUFT code to simulate tracer **transport** from different recharge sources (Figure 16). The three primary recharge sources near the dairy site are canal, dairy crop irrigation, and irrigation from surrounding areas. The transport simulation results indicate that nitrate entering the saturated zone from dairy crop irrigation is contained in the upper parts of the aquifer. Nitrate containment occurs within the high permeability sand-dominated perched aquifer because the dairy irrigation wells screened in the perched aquifer effectively capture nearly all recharge from dairy crop irrigation. The dairy irrigation wells pump groundwater at rates far higher than the recharge from dairy crop irrigation. The dairy irrigation wells also extract groundwater originating from irrigation of surrounding areas, canal leakage, and older groundwater

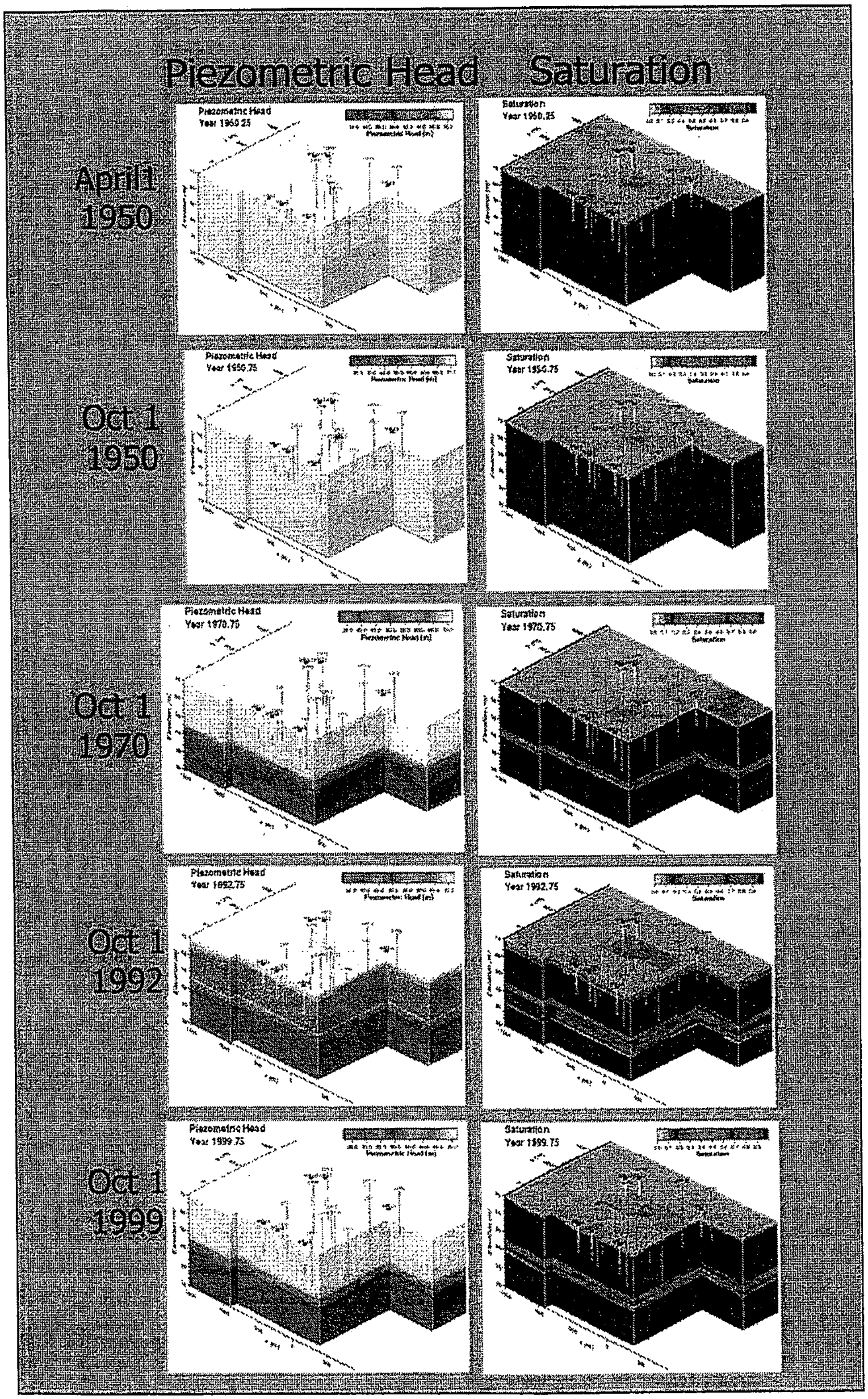

Figure 15. Simulation of groundwater flow at KCD1.

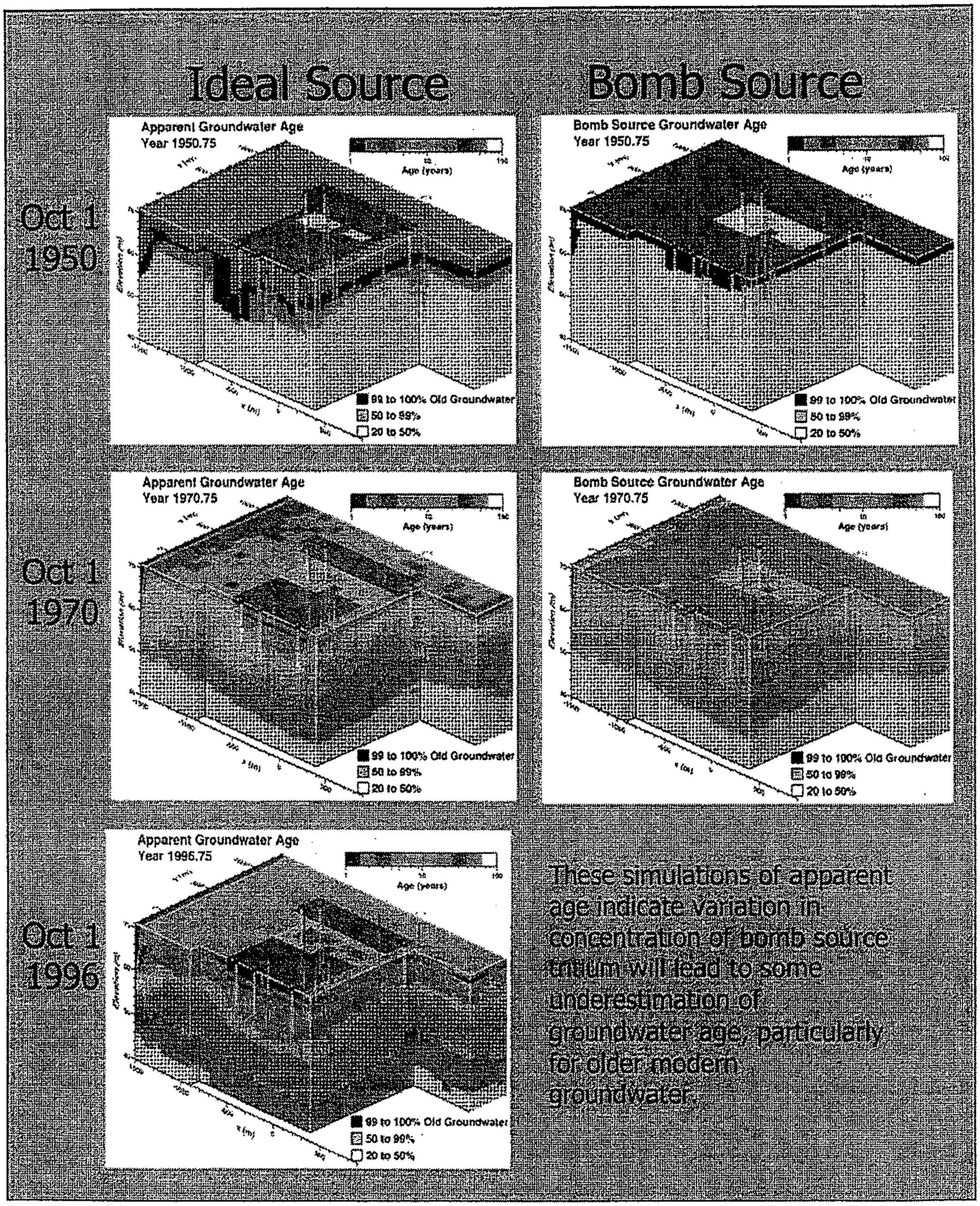

Figure 17. Simulation of apparent groundwater age at KCD1.

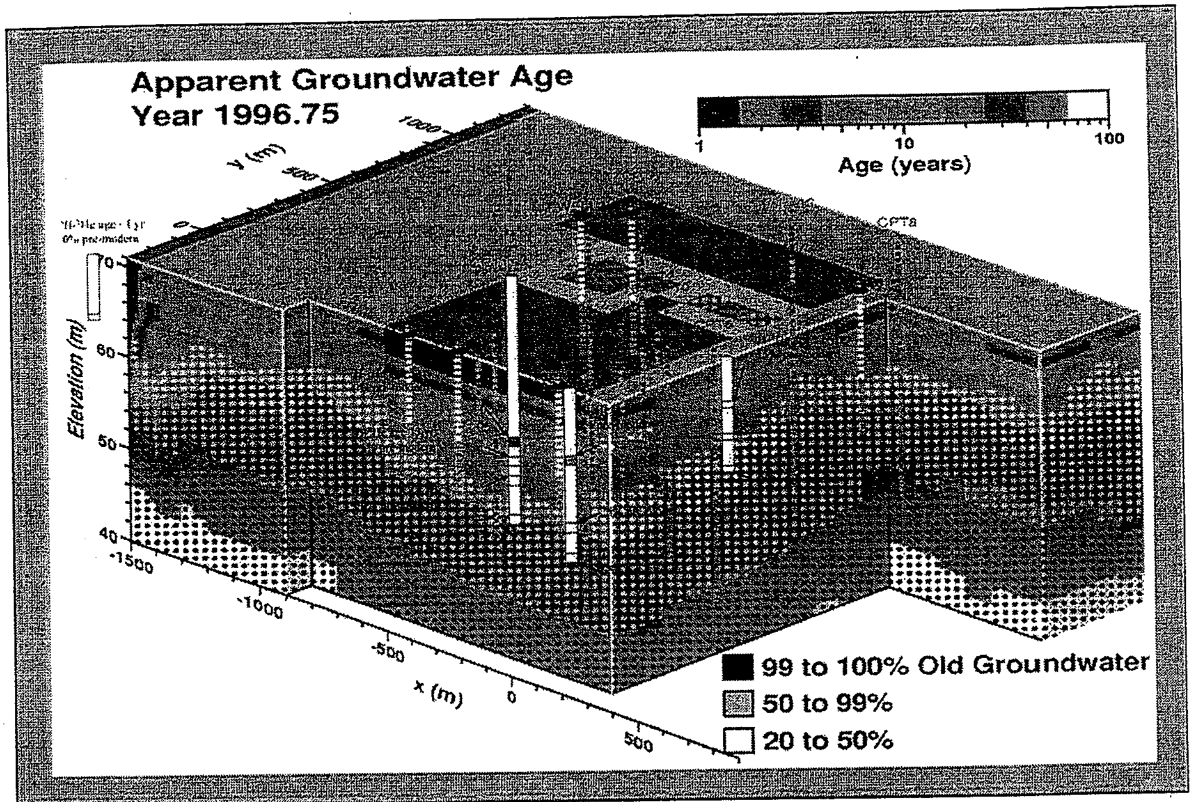

Figure 18. Comparison of measured and simulated groundwater ages at KCD1.
Agreement between measured and simulated apparent groundwater age at KCD1. See text for explanation.

The simulation of apparent age show excellent agreement for the southern Site 1 and Site 4 wells south of the dairy operation (Figure 18). At these well cluster locations, simulated ages are less than measured tritium/helium-3 ages in shallow groundwater at these sites because the simulations assumed that ^{3}He begins accumulating at the ground surface and not the water table. Current modeling efforts address this effort and produce better agreement for shallow groundwater. At Site 2 to the southeast of the dairy operation, measured groundwater ages are younger than simulated ages. This difference may indicate the absence of a shallow clayey zone at this location. These simulations of apparent age indicate variation in concentration of bomb source tritium will lead to some underestimation of groundwater age, particularly for older modern groundwater.

Conclusions. Coupling flow and transport simulations with groundwater age data and geostatistical simulations of hydraulic properties provides invaluable insights. Heterogeneity plays a large role in creating the perched aquifer and in causing vertical compartmentalization of flow patterns. The hydrofacies architecture consists of laterally continuous sand with interbeds of silt and clayey zones. Maintaining head and saturation in perched zone requires a continuous ~3 foot-thick clay layer at ~ 85 feet bgs. Flow simulation desaturates upper portions of the deep zone below the confining layer, and is consistent with observation of de-saturated zone below ~ 80 feet bgs.

The perched zone draws older water and recharge mostly from irrigation and less so from canal leakage. The dairy site pumps more groundwater from the perched aquifer than is recharged by crop irrigation, and thus physically contains lateral and vertical migration of nitrate contamination. High nitrate irrigation water penetrates to depths below the sharp redox gradient. Without denitrification, nitrate concentrations would be greater below the redox gradient, as is consistent with the presence of excess nitrogen in this zone.

The NUFT model presented here does not simulate transport of reactive constituents such as oxygen, nitrate, sulfate and organic carbon, and does not directly address the sharpness and uniform depth of the redox gradient in the shallow groundwater system. The strong vertical compartmentalization of the groundwater flow created by agricultural pumping and the location of the redox gradient close to the top of the production well screens, however, suggest that agricultural pumping and lateral groundwater flow may be important controls on the development of redox stratification in the shallow aquifer.

The Development of Reducing Conditions in Dairy Site Groundwaters

At three sites in this study (KCD, SCD, and MCD), dairy operations have been demonstrated to impact groundwater quality. At all three sites, nitrogen mitigation (either through denitrification or denitrification) has been demonstrated in groundwater impacted by manure lagoon seepage, a finding consistent with geochemical reactive transport modeling. At two of the sites (KCD and MCD), denitrification has also been demonstrated to occur in deeper waters impacted by irrigation with dairy wastewater. For denitrification to occur in the saturated zone, dissolved oxygen must be absent or present in very low concentrations. A key question, then, in assessing the ability of a groundwater to assimilate nitrate loading is what mechanism drives the development of reducing conditions necessary for denitrification to occur.

At the best studied site, KCD1, evidence exists for both natural and anthropogenic influence on the development of suboxic and anoxic groundwater. The deep aquifer at the KCD1 site consists of old water un-impacted by agricultural inputs. The water is tritium-dead and has a radiogenic ^4He age of approximately 100 years. In addition to having a mean age that pre-dates the intensification of agricultural activities, especially with regards to fertilizer usage and manure production, the deep aquifer groundwater has a chemical composition that indicates the absence of significant agricultural input. Salinity, dissolved organic C, nitrate and excess nitrogen are all low. This water is also anoxic, with nondetectable dissolved oxygen, detectable hydrogen sulfide, and low ORP. The electron donor responsible for reducing conditions is not known. Groundwater DOC is low, as is sediment solid-phase total S and organic C. Reduced sediment phases, however, are sufficient to create reducing conditions, even for slow redox processes such as solid-phase autotrophy given the age of the water. These observations all indicate that regionally reducing conditions un-related to agricultural activities do exist at the KCD1 site. Rates of denitrification in this deep system are unconstrained but may be slow and controlled by the abundance or reactivity of solid-phase electron donors.

The perched shallow aquifer is impacted by agricultural operations. Total inorganic nitrogen ($NO_3 + NO_2$ + excess N_2) shows a secular trend with apparent groundwater age, with the highest concentrations in the youngest water. The isotopic composition of high-nitrate waters indicates a wastewater source. Groundwater transport modeling indicates that irrigation dominates recharge in the perched aquifer. Irrigation with dairy wastewater results in the percolation of high-nitrate water to the water table and the penetration of this water to a depth controlled by agricultural pumping (Figure 16). Both the vertical and later transport of irrigation water is controlled by agricultural pumping. The perched aquifer is also strongly stratified with respect to oxidation state, nitrate distribution, and denitrification activity. Denitrification under irrigated fields occurs where oxic high-nitrate irrigation water mixes with older anoxic water. The mixing or "reaction" zone is sharp and at constant depth, and may be controlled by agricultural pumping.

What is the electron donor for the denitrification observed at the oxic-anoxic interface? Sediment organic-C and total-S concentrations in the deep and perched aquifer are comparable and are sufficient (assuming most of the S to be present in reduced phases) to create reducing conditions and support denitrification. At one shallow site (Site 3) upgradient of the main dairy operation, PCR data do indicate the presence of autotrophic bacteria capable of using reduced S as an electron donor, and geochemical modeling is consistent with pyrite oxidation. This evidence is not seen at the other sites, however, and the vertical variability in sediment C and S, does not explain the sharpness or location of the oxic-anoxic interface. Total organic carbon in site groundwaters varies from < 1 to 20 mg/L. (Neither other potential dissolved-phase electron donors such as thiosulfate nor the reactivity or bioavailability of the dissolved organic carbon was characterized.) Geochemical modeling is consistent with organic C oxidation, although simple models that assume shallow and deep waters have similar initial chemical compositions do not match observed compositions tightly. These observations, coupled with the lack of evidence for widespread distribution of autotrophic denitrifying bacteria in active denitrification zones, indicate that heterotrophy dominates the observed denitrification in the agriculturally-impacted perched aquifer. Simulations of irrigation and pumping at the KCD1 site indicate that groundwater flow at this site is strongly vertically compartmentalized. Te location of the redox gradient close to the top of the production well screens suggests that agricultural pumping and lateral groundwater flow in conjunction may be important controls on the development of chemical and redox stratification in the shallow aquifer.

The conceptual model, then, is of a regionally extensive deep aquifer that is naturally reducing and is unimpacted by agricultural operations overlain by a shallow aquifer that in its upper strata is strongly stratified, is reducing, and is the site of active denitrification of dairy-derived nitrate, and that these conditions in the shallow aquifer are driven by irrigation with dairy wastewater and groundwater pumping for dairy operations. This proposition, that denitrification in shallow nitrate-impacted aquifers is driven by dairy operations, is consistent with observations at not only the KCD1 site but also with evidence for denitrification at the MCD and SCD sites. The implication is that to assess net impact of dairy operations on groundwater quality, one must consider denitrification in the saturated zone.

CONCLUSIONS

The three primary findings of this research are that dairy operations do impact underlying groundwater quality in California's San Joaquin Valley, that dairy operations also appear to drive denitrification of dairy-derived nitrate in these groundwaters, and that new methods are available for characterization of nitrate source, transport and fate in the saturated zone underlying dairy operations.

Groundwater quality impact has been demonstrated at three sites, with a site in the southern San Joaquin Valley, KCD1, being the best characterized. High nitrate in groundwaters underlying these dairy sites can be attributed to dairy operations using a number of methods, including

- Chemical composition and nitrogen speciation.
- Nitrate isotopic composition.
- Groundwater dissolved gas content and composition.
- Groundwater age
- Reactive transport and flow modeling

The use of chemical composition, nitrogen speciation, and nitrate isotopic composition are well described in the literature. The use of dissolved gas content to identify manure lagoon seepage is new, and is introduced in this research. Groundwater age and transport simulations can be used to trace contaminants back to their source.

In both northern and southern San Joaquin Valley sites, saturated-zone denitrification occurs and mitigates the impact of nitrogen loading on groundwater quality. At the southern KCD1 site, the location and extent of denitrification in the upper aquifer is driven by irrigation with dairy wastewater and groundwater pumping. The extent of denitrification can be characterized by measuring "excess" nitrogen and nitrate isotopic composition while the location of denitrification can be determined using a PCR bioassay for denitrifying bacteria that developed in this research. The demonstration of saturated-zone denitrification in dairy groundwaters is important in assessing the net impact of dairy operations on groundwater quality.

New tools available for research on dairy groundwater include the determination of groundwater dissolved gas content to distinguish dairy wastewater irrigation from dairy wastewater lagoon seepage, field determination of excess nitrogen to identify denitrification in synoptic surveys and to characterize the extent of denitrification in monitor and production well samples, bioassay of aquifer sediment and water samples for the presence of denitrifying bacteria, characterization of aquifer heterogeneity using direct-push drilling and geostatistical simulation methods. Application of these new methods in conjunction with traditional hydrogeologic and agronomic methods will allow a more complete and accurate understanding of the source, transport and fate of dairy-derived nitrogen in the subsurface, and allow more quantitative estimates of net impact of dairy operations on underlying groundwater.

PUBLICATIONS AND PRESENTATIONS

Peer-Reviewed Presentations

McNab W. W., Singleton M. J., Moran J. E., and Esser B. K. (2007) Assessing the impact of animal waste lagoon seepage on the geochemistry of an underlying shallow aquifer. *Environmental Science & Technology* **41**(3), 753-758.

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Esser B. K., Beller H. R., Carle S. F., Hudson G. B., Kane S. R., LeTain T. E., McNab W. W., and Moran J. E. (2005) New approaches to characterizing microbial denitrification in the saturated zone (abstr.). *Geochimica et Cosmochimica Acta* **69**(10), A229. 15th Annual Goldschmidt Conference (Moscow, ID, May 20-25, 2005).

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Table 1: KCD2, KCD3, & SCD Site Data

Field Parameters, chemical compositon, groundwater age, recharge temperature, excess air, stable isotopic composition, excess nitrogen

(Unless otherwise indicated, all analytes are reported as mg/L; nitrate is reported as nitrate)

Name	Collection date	pH	DO	TOC	Na^+	K^+	Ca^+	Mg^{++}	Cl^-	SO_4^{--}	NO_3^-	NO_2^-	NH_4^+	excess N_2 (NO_3^- equiv)	Br^-	F^-	Li^-	PO_4^-	$^3H/^3He$ age (yr)	Recharge T (°C)	Excess air (cc STP/g)	H_2O-$\delta^{18}O$ (‰ SMOW)	NO_3^--$\delta^{15}N$ (‰ Air)	NO_3^--$\delta^{18}O$ (‰ SMOW)
KCD2 DW-1	2005/04/26	8.2	0.2		105	1	10	0	64	41	7	0.11	<0.02	2	0.21	0.06	0.005	0.99		15	8.8E-03	-11.1		
KCD3 DW-1	2003/08/21				87	0	54	1	134	57	9	1.22	nd		0.05	0.14	nd					-11.7	17.7	10.6
SCD1 Y-03	2005/03/08	6.8	0.6	18	215	4	124	55	59	199	185	0.41	<0.02	37	0.36	0.11	0.007	<0.04			2.5E-01	-9.8		
SCD1 Y-10	2005/03/08	7.0	5.3	3	82	137	110	81	143	16	42	1.31	137	nd	0.54	0.17	0.008	<0.04		18	9.8E-04	-9.1		
SCD1 Y-13	2003/08/26	7.5			28	5	146	41	48	169	58		<0.02		0.15	0.43	0.005	0.22	>50	16	2.0E-02	-11.0		
SCD1 Y-14	2003/08/26	7.3			63	5	146	55	57	233	167	0.05	<0.02	nd	0.12	0.26	0.003	0.22				-11.5		
SCD1 Y-15	2003/08/26	7.3			50	5	44	54	50	98	62	0.01	<0.02		0.12	0.23	0.006	0.24				-9.7		
SCD1 Y-16	2003/08/26	7.0			48	3	181	43	34	172	201	0.02	<0.02	nd		0.07	0.009	0.29	9	17	1.4E-02	-10.3		
SCD1 Y-17	2003/08/26	7.2			145	6	223	69	75	488	178		<0.02	nd	0.40	0.15	0.004	0.24	9		1.6E-03	-10.5		
SCD1 Y-18	2003/08/26	7.1			132	7	138	45	52	205	207	0.07	<0.02	nd		0.17	0.009	4.44	8	17	8.0E-03	-9.6		

Table 2: KCD1 Site Sediment C, S Data

KCD well cluster	Texture	Depth (ft)	Total C (wt%)	Tot C (2sd)	Carb C (wt%)	Carb C (2sd)	Org C (wt%)	Org C (2sd)	Total S (wt%)	Total S (2sd)	Sulfate S (wt%)	Sulfate S (2sd)	Reduced S (wt%)	Reduced S (2sd)
Site 1	Silty Sand	18	0.079	0.008	0.007	0.002	0.072	0.008	0.057	0.006	0.054	0.011		
Site 1	Clayey Silt	21	0.065	0.007			0.065	0.007	0.009	0.004				
Site 1	Sandy Silt	24	0.042	0.005			0.042	0.005	0.011	0.004				
Site 1	Clayey Silt	26	0.044	0.005			0.044	0.005	0.013	0.004				
Site 1	Sand	33	0.064	0.006			0.064	0.006	0.012	0.004				
Site 1	Sand	38	0.138	0.014	0.006	0.002	0.132	0.014	0.011	0.004	0.017	0.011		
Site 1	Sand	48	0.108	0.011	0.002	0.001	0.107	0.011	0.070	0.007	0.022	0.011	0.047	0.013
Site 1	Silt	61	0.050	0.005			0.050	0.005	0.011	0.004				
Site 1	Sandy Silt	69	0.066	0.007			0.066	0.007	0.022	0.004	0.019	0.011		
Site 1	Silty Sand	76	1.299	0.130			1.299	0.130	0.155	0.016	0.077	0.011	0.078	0.019
Site 1	Sand	77	0.207	0.021			0.207	0.021	0.181	0.018	0.034	0.011	0.147	0.021
Site 1	Sandy Silt	171	0.074	0.007	0.011	0.002	0.064	0.008	0.012	0.004	0.019	0.011		
Site 1	Sand	178	0.072	0.007	0.003	0.002	0.069	0.007	0.016	0.004	0.015	0.011		
Site 1	Silt	185	0.037	0.005			0.037	0.005	0.025	0.004				
Site 2	Sand	16	0.101	0.010			0.101	0.010	0.012	0.004				
Site 2	Sand	21	0.107	0.011			0.107	0.011	0.009	0.004				
Site 2	Silt	22	0.040	0.005			0.040	0.005	0.010	0.004				
Site 2	Sandy Silt	26	0.036	0.005			0.036	0.005	0.009	0.004				
Site 2	Sand	31	0.061	0.006			0.061	0.006	0.009	0.004	0.017	0.011		
Site 2	Clayey Silt	32	0.052	0.005			0.052	0.005	0.010	0.004				
Site 2	Sand	37	0.037	0.005			0.037	0.005	0.010	0.004	0.022	0.011		
Site 2	Sandy Silt	41	0.080	0.008			0.080	0.008	0.007	0.004				
Site 2	Sand	43	0.028	0.005			0.028	0.005	0.012	0.004	0.020	0.011		
Site 3	Sandy Silt	11	0.043	0.005			0.043	0.005	0.011	0.004	0.021	0.011		
Site 3	Silt	14	0.035	0.005			0.035	0.005	0.011	0.004				
Site 3	Sandy Silt	17	0.045	0.005			0.045	0.005	0.041	0.007	0.038	0.005		
Site 3	Sand	20	0.083	0.008			0.083	0.008	0.011	0.004				
Site 3	Sand	27	0.080	0.008			0.080	0.008	0.015	0.004				
Site 3	Sand	32	0.147	0.015	0.014	0.002	0.132	0.015	0.025	0.004	0.035	0.011		
Site 3	Sand	36	0.073	0.007	0.004	0.002	0.068	0.007	0.019	0.004	0.023	0.011		
Site 3	Sand	40	0.059	0.006	0.002	0.001	0.057	0.006	0.018	0.004	0.016	0.011		
Site Temp	Clayey Silt	5	0.187	0.019			0.187	0.019	0.010	0.004	0.019	0.011		
Site Temp	Clayey Silt	8	0.107	0.011	0.001	0.001	0.106	0.011	0.008	0.004	0.016	0.011		
Site Temp	Clayey Silt	8	0.181	0.018			0.181	0.018	0.020	0.004	0.015	0.011		
Site Temp	Sandy Silt	14	0.070	0.007			0.070	0.007	0.009	0.004	0.023	0.011		
Site Temp	Clayey Silt	16	0.058	0.006			0.058	0.006	0.011	0.004	0.021	0.011		
Site Temp	Clayey Silt	23	0.035	0.005			0.035	0.005	0.008	0.004	0.019	0.011		
Site Temp	Sand	27	0.029	0.005			0.029	0.005	0.007	0.004	0.017	0.011		
Site Temp	Clayey Silt	28	0.050	0.005			0.050	0.005	0.008	0.004				
Site Temp	Sand	36	0.057	0.006	0.003	0.002	0.053	0.006	0.008	0.004	0.016	0.011		

Table 3. KCD1 Sediment PCR Data

KCD1 Well Cluster	Depth (ft)	Total *Nir* (gene copies/ 5 g sediment)	Total eubacteria (cells/ 5 g sediment)
Site 1	21	7.9E+03	1.1E+06
Site 1	27	nd	3.9E+06
Site 1	29	1.1E+04	1.0E+06
Site 1	30	5.1E+03	3.9E+05
Site 1	32	3.8E+03	1.9E+06
Site 1	36	1.1E+05	6.7E+06
Site 1	45	9.5E+03	6.9E+05
Site 2	29	9.6E+04	2.0E+06
Site 2	31	1.1E+04	5.4E+05
Site 2	34	1.6E+05	3.8E+06
Site 2	36	2.8E+05	1.2E+07
Site 2	38	2.2E+07	1.7E+08
Site 2	40	1.3E+06	1.9E+07
Site 2	44	5.6E+03	1.4E+05
Site 3	30	6.6E+03	5.9E+05
Site 3	38	3.6E+04	9.6E+05
Site 3	40	3.4E+04	2.6E+06
Site 3	42	9.6E+04	2.1E+06
Site 3	44	3.7E+04	7.4E+05
Site 3	46	1.9E+05	7.5E+06
Site 3	48	1.4E+05	6.9E+06
Site 4	28	2.5E+04	6.9E+05
Site 4	33	3.0E+04	1.1E+06
Site 4	43	1.9E+05	1.8E+06
Site 4	45	9.1E+04	4.9E+05
Site 4	47	7.2E+04	5.2E+05
Site 4	49	4.6E+04	1.7E+06